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LEAFSPOT OF MAIZE CAUSED BY OPHIOBOLUS HETEROSTROPHUS, N. SP., THE ASCIGEROUS STAGE OF A HELMINTHOSPORIUM EXHIBITING BIPOLAR GERMINATION[1]

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INTRODUCTION

For several years the writer has received for identification occasional lots of graminaceous material attacked or suspected of being attacked by members of the genus Helminthosporium. Among the more noteworthy of these are certain collections of maize (*Zea mays* L.) leaves affected with a foliar disease, which, with the parasite responsible for it, this writer has briefly described in an abstract (*3*)[2] and which is more adequately treated in this paper. While the fungus has evidently not escaped observation by previous writers, it has generally been confused with a congeneric form responsible for leaf blight of maize. Additional interest attaches to the fungus under consideration in that it readily yields an ascigerous stage of a type not hitherto recorded as associated with any species of Helminthosporium. The discovery of this association is presumably not without significance in relation to the taxonomic position of a numerous series of apparently allied forms assigned to the genus, including, for example, the parasites causing spotblotch of barley, eyespot of sugar cane, and leafspot of rice, of which the mode of conidial germination by the production of a polar germ tube from one or both end segments represents a common characteristic.

SYMPTOMS

Liberal collections of diseased maize leaves made by A. C. Foster near Sanford, Fla., on September 22, 1923, and by C. Welles near Los Baños, Philippine Islands, in November, 1921, very largely provided the material upon which the present studies were based. The leaves bore numerous lesions, varying considerably in extent, the smaller and evidently incipient ones scarcely discernible and measuring approximately 0.5 mm. in each direction, the larger up to 15.0 mm. in length and from 1.0 to 3.0 mm. in width (fig. 1, A). In the dried condition the interior of the larger spots approximated, on the whole, the cinnamon-buff of Ridgeway's color chart, although this hue was interrupted by rather delicate transverse reddish-brown bands at intervals of 1.0 to 1.5 mm., resulting in a perceptibly zonate appearance. Reddish-brown coloration was equally present also at the margin of the lesion, setting off the latter from the healthy tissue

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[2] Reference is made by number (italic) to "Literature cited," p. 726.

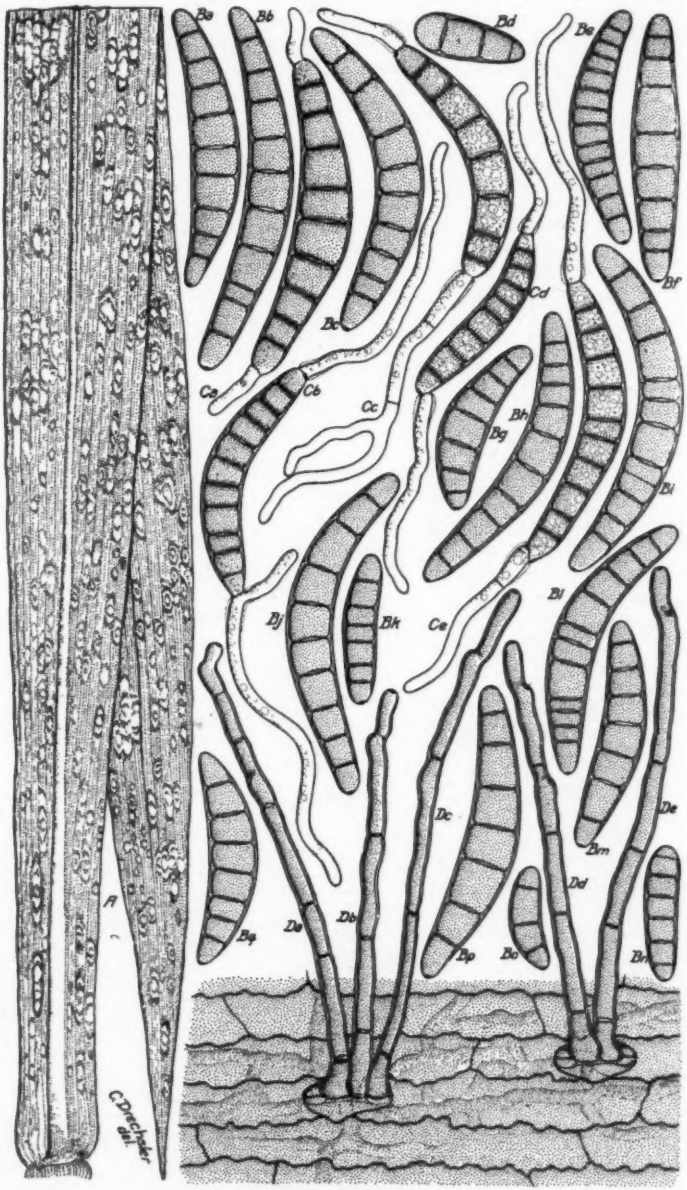


FIG. 1.—A, leaf of maize infected with *Ophiobolus heterostrophus*, showing abundance and size of lesions in specimens collected near Sanford, Fla., Sept. 22, 1923. ×7
Ba–Bq, conidia of *O. heterostrophus* produced on diseased maize leaves in damp chamber at summer temperature (25 to 30° C.) × 474
Ca–Ce, conidia germinating by production of 2 polar germ tubes. × 474
Da–De, conidiophores emerging from stomata as found on material collected in the field. × 474

surrounding it. On some of the less mature foliage a purple tint was frequently evident in lesions that approached the Prussian Red of Ridgway's series. All except the very smallest lesions were elongated in a longitudinal direction, usually with the lateral edges characteristically straight and parallel and coinciding accurately with the course of the veins. Since the latter in the Florida material were spaced at intervals of 1.0 to 1.2 mm., and in the Philippine specimens at intervals of 2.0 to 3.0 mm., the lesions appeared generally as ribbonlike areas of corresponding width. In occasional instances two adjacent intervascular spaces were included in the dead regions and these consequently were double the usual width. The larger regions of discolored foliar tissue, however, had their origin less frequently in such lateral extension of single lesions than in the coalescence of numbers of lesions, side by side and end to end. Owing to the abundance of the spots, which numbered from 200 to 300 on leaves not more than 50 cm. long and 2.5 cm. wide, the morbid parts naturally constituted a considerable portion of the foliar tissue.

Under macroscopic inspection, the appearance of the leaves thus affected does not closely resemble that of specimens affected with blight, a well-known disease widely distributed in maize-producing countries and caused by *Helminthosporium turcicum* Passerini. The blight lesions are relatively few in number, usually not exceeding a dozen to an individual leaf, but their paucity is more than compensated for by their size; they are, when well developed, more than a hundred times larger than even the largest seen in the Florida material. A comparison of Figure 1, A, with Plate 24, A, in a previous publication of the writer (*2*) suggests the difference in number and size of the two types of lesions. Leaf-blight lesions show no indication of zonation other than a reddish-brown marginal border which often is not well developed or is even entirely suppressed. And while the portions of leaf killed by *H. turcicum* are also elongated longitudinally and often bounded on the sides by the veins of the host, more than one intervascular region are usually involved, the veins apparently providing a much less effective barrier to lateral extension than is evidenced in the specimens under consideration.

CONIDIAL STAGE OF THE CAUSAL PARASITE

Macroscopic examination revealed conidiophores and conidia typical of the genus Helminthosporium scattered rather sparsely over the central portions of the larger lesions, the constant positional association strongly suggesting the causal relation subsequently established by inoculation experiments. On the material as obtained from the field, the conidiophores exhibited no unusual peculiarity. Singly, or in groups of two or three, they were found to emerge from the stomata (fig. 1, *Da* to *De*), resembling in the latter detail the homologous structures of *H. turcicum;* although such limitation to stomata is regarded as due to the mechanical firmness of the maize epidermis rather than to an inherent characteristic of the parasites concerned. However, a conspicuous difference distinguishing them from the conidiophores of the leaf-blight fungus was their smaller diameter, in which dimension they were found to measure usually from 4.5 to 7.0 μ instead of 7.5 to 9.0 μ recorded for the other form. In length the conidiophore of the field material measured between 120 and 170 μ, while scars marking the former points of attachment

of spores occurred at fairly wide intervals (10 to 40 μ) at geniculations usually not very pronounced.

Owing to the meager development of the fungus on the material obtained in the field, some of the diseased leaves were incubated in a damp chamber. A luxuriant growth of fructifications at once ensued, with the result that at the end of three days the leaves were covered with a profusion of conidiophores bearing an abundance of fresh conidia (fig. 1, *Ba* to *Bq*). Pure, single-spore cultures of the fungus were obtained by the usual method of transferring individual conidia germinating in poured plates of plain agar to cornmeal agar plates, and then transferring portions of the resultant growth, free of contaminating forms, to cornmeal agar in tubes.

The conidiophores developed in a moist atmosphere on the natural substratum, as in related forms, are not sharply differentiated from the sterile hyphae. Usually they consist of branches or prolongations of subhyaline or fuliginous mycelial filaments, differing from the latter in having a thickened wall dark olivaceous in color. As on the conidiophores found in nature, they develop spores in rather open arrangement, the individual conidia being attached at relatively wide intervals. Branching is not rare although it is more frequent in the sterile portions of the sporiferous elements. The sporophoric filaments, which often attain a length of 1 mm. or more, present, in mass, a brownish-black macroscopic appearance which may become brownish gray or even light gray, depending upon the quantity of subhyaline vegetative mycelium present as an admixture.

The conidia of the fungus as produced on diseased maize leaves in a damp chamber (fig. 1 *Ba* to *Bq*) or on cornmeal agar in pure culture (fig. 2), are readily distinguished from those of *Helminthosporium turcicum* in shape, dimensions, septation, and basal modification. While the spores of the leaf-blight fungus are typically straight or slightly curved, widest at the middle and tapering markedly toward the ends, those of the leafspot parasite are typically strongly curved, the curvature being indeed more pronounced than in any large-spored congeneric form studied by the writer, and tapering moderately toward the ends. In respect to size, the conidia of the new foliar parasite appear markedly inferior to those of the other, measuring only 30 to 115 μ in length and 10 to 17 μ in diameter as compared with 45 to 142 μ and 15 to 25 μ for the ranges of the corresponding dimensions in *H. turcicum*. In spite of its inferior length, however, the conidia of the leafspot fungus are the more abundantly septate, the number of cross-walls not infrequently reaching the observed maximum of 12, while in all material of *H. turcicum* examined by the writer, these have never been seen to exceed 8. An additional difference exists, inasmuch as in the fungus under consideration the hilum is represented by a broad flat scar not generally protruding from the rounded contour of the basal end of the conidium, while the basal segment of the spore of *H. turcicum* exhibits a narrow protruding apiculum. With respect to coloration the distinction is less marked, although the light or moderate olivaceous color characteristic of the mature conidia of the leafspot fungus is noticeably darker than the fuliginous tinge generally present in the conidia of the leaf blight parasite. The same mode of germination by the production of two germ tubes, one from the apical end, the other from the region surrounding the hilum, prevails in both species (fig. 1, *Ca* to *Ce*).

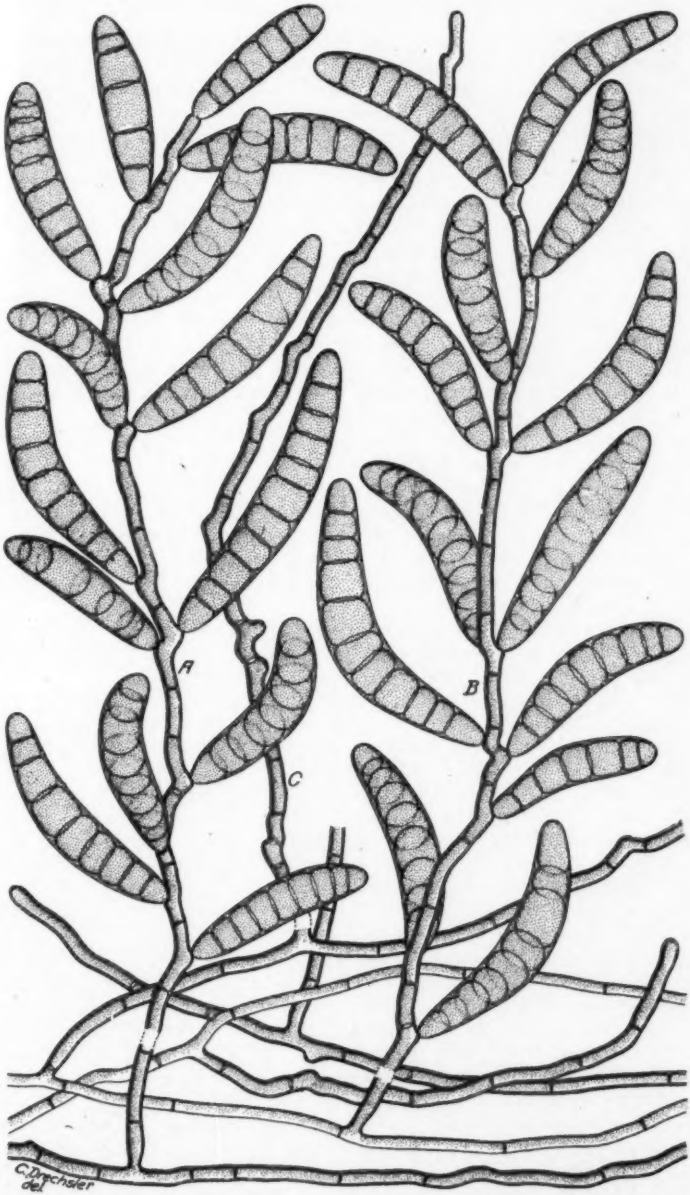


FIG. 2.—Conidiophores and conidia of *Ophiobolus heterostrophus* developed on 30-day-old cornmeal agar plates, arising from somewhat prostrate sporophoric filaments. The dotted lines represent sections of the sterile basal portions of the sporophores omitted for lack of space. ×475

DISTRIBUTION ON MAIZE

In this connection, it may not be amiss to consider some passages in the writings of several investigators working on leaf blight in tropical or subtropical regions, which can scarcely be regarded as aptly descriptive of *Helminthosporium turcicum*. Reinking (*10*), working in the Philippines, described the conidia of *H. inconspicuum* C. and E. (=*H. turcicum* Pass.) as "large, septate, curved brown," a characterization that in regard to coloration and curvature is not generally appropriate. Two figures—one (*10, fig. 40 B*) representing a conidium from a maize tassel, the other (*10, fig. 40, lower c*) representing a conidium from a maize leaf—especially deserve comment. The former is shown as a slightly curved structure with nine cross-walls, diminishing only slightly toward the abruptly rounded end, and, from calculations of the size of the figure and the scale of magnification, measuring approximately 112 μ in length and 17 μ in diameter; the latter is more strongly curved, provided with 10 septa, tapering somewhat more strongly toward the rounded ends, and measuring approximately 128 μ in length by 17 μ in diameter. The spores would appear to be too slender relative to their length, too abundantly septate, and not tapering toward the ends sufficiently to be typical of the leaf-blight fungus. Of the other figures given by the same author, *41b* would evidently seem to represent conidia of *H. turcicum*, while *40 upper c* as well as *43* appear to pertain to this species, though with less certainty. And the habit figure undoubtedly represents the extensive foliar lesions characteristic of leaf blight.

The descriptive passages in Marquez' recent paper (*7*) on leaf blight of corn, incorporating the results of studies carried out at Los Baños, are not free of the same sort of ambiguity.

Since the lesions of both leaf blight and leafspot are elongated in a longitudinal direction and are apt to coalesce, the description of the earlier stages may have been drawn from specimens representing either type or probably both types of infection. The leafspot lesion with its parallel lateral boundaries coinciding with two consecutive veins of the host are more accurately described as "stripes" than the effuse areas killed by leaf blight. They also coalesce with much greater frequency, indeed not attaining any considerable size unless abundant coalescence takes place, since the greatest individual lesions rarely exceed 3.0 mm. in width and 15.0 mm. in length. The leaf blight lesions on the other hand, become relatively very extensive without any coalescence whatever. It is not improbable that the description of the old spots refers to the latter type of lesion, since fructifications of *Helminthosporium turcicum* are always visible as a grayish efflorescence in the central portion of large affected regions. In the material from both Florida and the Philippines as obtained from the field, the fructifications on the leafspot lesions have been too sparse to be macroscopically visible. Since, however, on incubation under moist conditions a copious production of sporulating fructifications ensues, it might be wrong to assume that the "black mold" described by Marquez could not possibly have been produced by the leafspot parasite. The total of microscopic detail given by Marquez is equally difficult to reconcile with the morphology of either species alone. From 20 measurements of conidiophores he found these structures to vary from 96 to 148 μ in length and from

8 to 10 μ in diameter; while 200 spores varied from 36 to 112 μ in length, and from 10 to 18 μ in diameter. As the sporophores and spores of *H. turcicum* do not differ greatly in length from those of the leafspot fungus, the figures given for this dimension might be nearly equally acceptable for either species. On the other hand, the diameter of these structures differs considerably between the two parasites. The range given for the diameter of conidiophores would seem much more acceptable for *H. turcicum* than for the fungus causing leafspot; while the range in spore diameter is certainly characteristic of the leafspot parasite and not at all characteristic of *H. turcicum*. Marquez' statement that "the conidia are wider at the middle and gradually taper toward the two ends; they are septate, and are crescent-shaped" would seem to lend additional support to the assumption that he dealt with the leafspot fungus.

The inferences drawn from the publications of Reinking and of Marquez are altogether in harmony with direct evidence provided by examination of various lots of diseased material originating in the Philippines, mostly in the region in which those writers made their observations. The specimens of affected maize leaves collected near Los Baños in November, 1921, to which reference has already been made, exhibited a number of lesions typical of leaf blight, some of them 15 and 20 cm. long, with an abundance of fructifications in the center of the dead areas. The spores obtained from such areas differed in no wise from the conidia associated with leaf blight encountered in the Middle Atlantic States—broad, mostly straight, tapering markedly toward the basal end, provided with a protruding hilum, and with septa not exceeding eight in number. In addition, the leaves bore a much larger number of spots altogether similar to those present on the Florida material—similar in color and zonation as well as in being delimited laterally, when well developed, by the larger veins, which as the specimens represented more mature foliage, were spaced at intervals of approximately 3.0 mm. Preparations of the discolored areas revealed a relatively sparse development of conidiophores and conidia, which after treatment with chloral hydrate showed, except for the degenerate contents and swollen membranes characteristic of dead structures, complete similarity in morphological detail to the Florida fungus as collected in the field. The specific identity of the two forms was established beyond any question when some of the Philippine material was incubated in damp chambers. Some of the spots gave rise to an outgrowth of sporophoric filaments bearing conidia differing in no particular—size, septation, coloration, curvature, or basal modification—from those resulting from the incubation of Florida material under like conditions.

The fresh conidia were utilized in making a number of single-spore cultures, which again were completely like those of the Florida parasite. Complete similarity was found to obtain also in regard to perithecia, asci, and ascospores, as well as to the conidial fructifications developed in cultures derived from ascospores or pieces of immature perithecium. The fact that the material collected in November, 1921, gave rise to fresh fructifications when placed in a damp chamber as late as October, 1924, is indicative of a considerable measure of longevity in the mycelium of the fungus found within the tissues of the diseased leaves, since the old conidiophores and conidia could in no instance be made to germinate.

A specimen deposited in the herbarium of the Office of Pathological Collections bears the following label:

Philippine Fungi, Herbarium, College of Agriculture, Los Baños, Philippines No. 5. *Helminthosporium inconspicuum.* On leaf of Zea mays Linn. Los Baños. Laguna, P. I. Date, Dec. 17, 1919. Coll. L. Goco. Det. by O. A. Reinking.

This specimen was found to consist of a portion of maize leaf bearing numerous well-characterized leafspot lesions. A larger discolored region was also present which may have been a leaf-blight lesion, but if so, it had not developed far enough to exhibit the fructifications of *H. turcicum.*

In four collections of material made by W. H. Weston, jr., in the vicinity of Los Baños on July 17, 1918, September 10, 1918, September 18, 1918, and January, 1920, the injury observed could be assigned without difficulty and presumably with certainty, to the parasite under discussion, either alone or together with *Helminthosporium turcicum.* The first of the collections made in a field where three-fourths of the stand had been killed reveals both leafspot and leaf-blight lesions, the former scattered profusely everywhere over the foliar organs, the latter relatively few in number, but frequently very extensive, and often including numbers of leafspot lesions within their boundaries. The second collection made in a test plot where infection was described as rather serious, appeared entirely free of leaf blight, but exhibited an abundance of leafspot lesions. In the third collection, which was made in a field showing "noticeable loss," most of the leaves bore only leafspot lesions, while others were affected with leaf blight as well. On the young foliage that constitutes the collection of January, 1920, only leafspot lesions are evident, these being, however, both numerous and well developed. Although no general conclusions can be based on the few collections examined, it would appear that in 1918, 1919, 1920, and 1921 the leafspot disease occurred abundantly in the Philippines, manifestly being responsible for moderate losses, even in the absence of leaf blight, and, where combined with the latter malady, responsible apparently in large part for instances of more destructive injury.

In a brief discussion of Mitra's interesting paper on the disease of maize and sorghum attributable to *Helminthosporium turcicum* in India (*8*), attention was called to certain discrepancies in morphological detail between the account of the parasite given by that author and the account submitted by the writer from studies made in the Middle Atlantic States. It is believed that a partial explanation of these discrepancies may be found in the assumption that Mitra was dealing not only with the leaf-blight parasite, but with the leafspot fungus as well, notably in the Pusa and Malda specimens, which yielded spores described as "curved and narrow." Certainly some of the figures of spores from the Pusa maize leaves (*8, pl. II, 9, 10, 11, 13*) are more suggestive of the leafspot fungus than of *H. turcicum*, while the others (*8, pl. II, 7, 8, 12*) would seem to be more aptly illustrative of *H. turcicum.* It must be admitted, to be sure, that Mitra's statement that the lesions on the Pusa and Malda material were similar to those on specimens from other localities, and that cultures derived from this material bore spindle-shaped, straight, or rarely curved conidia does not lend support to such an explanation.

In the foregoing, attention has been confined to leafspot and leaf blight of maize, and the congeneric causal organisms responsible for them, without reference to the occurrence of Helminthosporium infection of the male inflorescence of maize, mentioned in several publications based on studies in the Tropics. Reinking in his discussion of *H. inconspicuum* (*10*), states that: "A black mold is produced on the tassel * * *. *Helminthosporium curvulum* Sacc. has been reported from the tassels of corn." In another paper (*11*) the same matter is restated as follows: "A black mold is also produced on the tassel. Saccardo has determined this fungus as *Helminthosporium curvulum* Sacc.; however, it produces a disease identical with the earlier described disease caused by *Helminthosporium inconspicuum*." In a host index of Philippine economic fungi, *H. curvulum* is enumerated among the fungi on corn as "tassel mold."

Mitra, in his description of maize blight, states that—

> In the male inflorescence the disease assumes a blackish mold-like appearance on the surface of the glumes of male spikelets. The attack is not extensive and scattered spikelets here and there are infected. The mycelium ramifies within the tissues of the paleae and the stamens.
>
> * * * Conidia of the fungus from the glumes of male spikelets are a little longer and more curved than those of the leaf fungus and has been named *H. curvulum* Sacc. In the Philippines it is described by this name but here in cultures it resembles the strain from the leaf and, when inoculated on the leaves, produces the same kind of spots as the leaf strain.

To these references may be added a passage from the writer's account (*2, p. 716*) of *Helminthosporium turcicum*, regarding the conidia obtained from affected maize tassels collected in the Philippines by W. H. Weston, jr.:

> Preparations made from the fructifications on the tassel, however, showed conidia which, while of the same color and approximate maximum length, were perceptibly inferior in diameter, measuring approximately 11 to 14 μ in this dimension; more abundantly septate, 12 transverse walls being not uncommon; usually quite distinctly curved; and evidently similar to those figured by Reinking (*10, pl. 20, B, C*). * * * If the forms on the leaves and on the tassels should indeed prove to be identical, the morphology of *Helminthosporium turcicum* as generally understood would stand in considerable need of revision.

Subsequent to the discovery of the leaf-spot disease, the writer took occasion to examine two packets of affected maize tassels deposited in the herbarium of the Office of Pathological Collections by C. F. Baker under the label:

> "*Fungi malayana* No. 239 *Helminthosporium curvulum* Sacc. n. sp. on *Zea mays*. Mount Maquiling, near Los Baños, Province Laguna, Philippines. Det. by Saccardo. Date Aug., 1914."

In general appearance the specimens closely resembled those collected by Weston, the fructifications where best developed being crowded on the scales as a velvety layer (fig. 3, F) very similar to the growth of *Helminthosporium oryzae* B. de H. on the inflorescence of rice. Microscopic examination revealed two species of Helminthosporium, one with relatively small sporophores (4 to 6 by 100 to 225 μ) and small 3-to-4 septate, curved conidia, measuring 9 to 14 μ in diameter by 20 to 30 μ in length, the middle cell of which was considerably smaller and darker than the other segments (fig. 3 *Ba to Bf, Ca to Cm*). The other fungus was apparently identical with the one found on Weston's material, the conidiophores measuring usually from 4.5

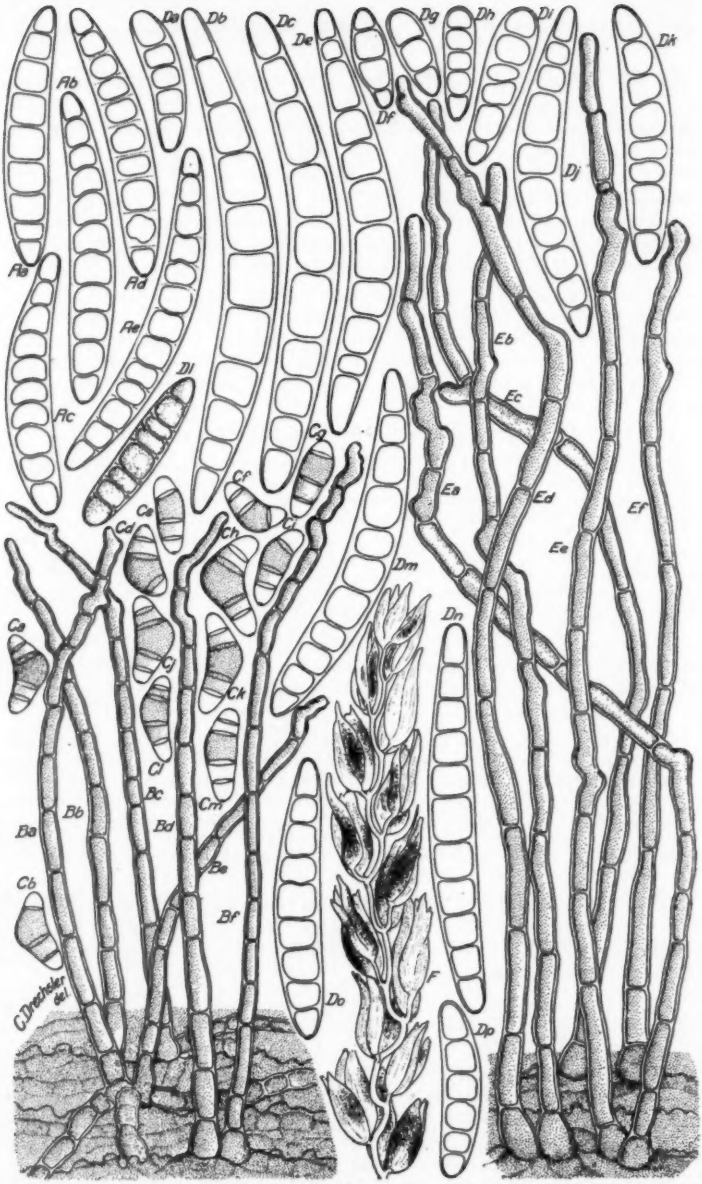


FIG. 3.—Aa-Ae. Conidia of *Ophiobolus heterostrophus* from specimen of maize leaf collected at Brooksville, Fla., June, 1917, deposited in herbarium of the Office of Pathological Collections. × 500

Ba-Bf. Conidiophores of the smaller species of Helminthosporium present on the specimen of maize tassels distributed as "Fungi Malayana No. 239," and evidently the one which Saccardo identified as H. curvulum Sacc. × 500

Ca-Cm. Conidia of the same species. × 500

Da-Dp. Conidia of the larger tassel mold present in the specimens distributed as "Fungi malayana No. 239," after treatment with chloral hydrate; not *H. curvulum*, nor *H. turcicum*, though perhaps *Ophiobulus heterostrophus*. × 500

Ea-Ef. Conidiophores of the foregoing species. × 500

F. Portion of infected tassel from "Fungi malayana No. 239" bearing a mixture of two species of Helminthosporium. × 0.1

to 8.0 μ in diameter, and sometimes more than 340 μ in length (fig. 3 *Ea to Ef*), while the light olivaceous, generally curved 2 to 12 septate conidia, gave as the more extreme measurements, 10 to 17 μ for diameter, and 28 to 155 μ for length (fig. 3, *Da to Dp*). When treated with chloral hydrate, the hilum appeared as a broad, rather inconspicuous scar included within the contour of the basal cell, not as a protruding modification. It was in all probability the latter fungus which Reinking and Mitra regarded as the leaf-blight fungus, and which apparently they believed Saccardo had identified as *H. curvulum*.

As to the correctness of a view holding the larger tassel mold identical with the leaf-blight fungus, no final decision is possible without a comparison of living material of both forms developed under similar conditions. In the absence of cultures of the larger species obtained from diseased tassels, the writer has been unable to make such comparison. He is, nevertheless, quite convinced that the two forms represent altogether distinct species—that, in short, the larger tassel mold is not *Helminthosporium turcicum*. The possibility remains, of course, that *H. turcicum* may occur on male inflorescences of maize to some extent, although the writer has not observed anything he could have identified with this species on any of the specimens examined by him.

In comparing the larger tassel mold with the leafspot fungus, a striking degree of similarity at once appears. In respect to diameter and spacing of septa in conidiophore and conidium, the two forms agree closely. Both are similar in that the conidia are strongly curved, are septate up to twelve times, are from 10 to 17 μ in diameter, and have a broad scar contained within the contour of the basal cell. The only evident difference is the length of conidiophore and conidia, these dimensions attaining considerably greater maxima in the material from affected tassels, although the difference between the general run of spores is not especially marked. It is possible that a condition prevails here similar to that found in the case of *Helminthosporium oryzae*, which produces, on the mats of well-developed sporophoric filaments that constitute the black mold on the inflorescence, conidia considerably longer than those produced on the poorly developed sporophores arising sparsely from the foliar lesions. Although the writer is inclined to regard the larger tassel mold on maize as identical with the parasite causing maize leafspot, he is not prepared to offer this view as a conclusive opinion.

In any case it appears certain that Reinking and Mitra were in error in assuming that the tassel mold which they regarded as identical with *Helminthosporium turcicum* was the one which Saccardo identified as *H. curvulum* Sacc., in spite of the aptness of the latter specific name. As the diagnosis of *H. curvulum* (*14, p. 89*) did not appear until 1919, Reinking probably drew a natural but mistaken inference from an examination of Baker's Fungi Malayana No. 239. The species is based apparently on a fungus on leaves of *Bambusa blumeana*, and in part is characterized as follows:

* * * Conidiophoris dense fasciculatis, filiformibus, 90–100=6–7μ, septatis, fuligineis, sursam pallidioribus obsoleteque denticulatis; conidiis obclavatis, distincte curvato-gibbis, 35–40=9–12, utrinque, praecipae basi rotundatis 3-septatis, non constrictis, grosse 4-guttatis, fuligineis, loculis binis mediis obscurioribus.

Unquestionably Saccardo's identification was concerned with the smaller species of Helminthosporium present on Baker's specimens as tassel mold, one of the several types frequently encountered when dead grass leaves are incubated in a damp chamber. In spite of its general distribution in nature, it appears to be of relatively little significance in relation to disease, and is discussed here only to clear up nomenclatorial confusion.

OCCURRENCE ON TEOSINTE

With the several lots of diseased maize leaves kindly sent by Weston were two liberal lots of teosinte (*Euchlaena mexicana* Schrad.) leaves collected in September and November, 1918, in the same locality as the maize collections. These leaves exhibit lesions strikingly similar to leafspot lesions on maize in shape and coloration, though minor differences are apparent in some instances in the less regularly straight lateral margins and the somewhat greater length of the lesions on teosinte, which may measure up to 3 cm. in a longitudinal direction (pl. 1, A, B). The lateral edges, in general, did not coincide as closely with the veins of the host as on maize. Microscopic examination revealed in the central parts of the dead areas, a sparse production of conidiophores and conidia, which when treated with chloral hydrate showed complete similarity to those on maize. Owing evidently to application of preservatives, efforts to recover the parasite in a living condition by incubating infected material in a damp chamber did not succeed. However, because of the close similarity in morphology and pathological habit, there can be little doubt that the same parasite is involved in the foliar disease of the two closely related hosts, which, it may be mentioned, have several other fungous parasites in common.

The occurrence of the leafspot fungus on teosinte raises the question as to the probable identity of the parasite found in the United States and in the Philippines with *Helminthosporium euchlaenae* described by Zimmerman (*17*) as pathogenic to this host in German East Africa. Zimmerman's description of the foliar lesions produced by the African form as light-brown spots with dark-brown margin, mostly elongated in a longitudinal direction, approximately 2 mm. wide and up to 25 mm. long, applies unusually well to the Philippine material. A not unsatisfactory agreement prevails also with reference to the sporophores which, according to the German account, are brown, unbranched, several times septate, 5 to 7 μ in diameter and up to 150 μ in length, and emerge from the stomata singly or in groups of two or three. The characterization of the conidia as brownish, cylindrical, straight or somewhat curved, up to seven times septate, 50 to 60 μ long and 13 to 15 μ in diameter, would seem to represent these structures as considerably inferior in degree of curvature, frequency of septation, and maximum length to the conidia on the Philippine specimens. If Zimmerman's text and figures give a sufficient description of the conidia of the African fungus, it would be necessary to conclude that the latter constitutes a species distinct from the Philippine form. However, with a fungus sporulating as sparingly as the leafspot parasite, the possibility of a description being based on a small number of conidia not altogether characteristic of the species is far from being a remote one. The question concerning the identity of the leafspot parasite with *H.*

A, B.—Portions of teosinte leaves showing lesions of *Ophiobolus heterostrophus*. Photographed from dried specimens originating from the vicinity of Los Baños, P. I., September, 1918.

C.—Portion of maize leaf showing numerous lesions of *O. heterostrophus* and a much larger lesion caused by *Helminthosporium turcicum* on the upper left margin. Photographed from dried specimens collected near Los Baños, P. I., July, 1918. × ⅔

D.—Portion of maize leaf showing numerous lesions of *O. heterostrophus* and a part of two moderately extensive lesions caused by *H. turcicum*, one immediately adjacent and to the right of the midrib, the other toward the left margin. The border of the second lesion is indicated by a dotted line for greater clarity. The cracks due to the brittle texture of tissues killed by *H. turcicum* indicate approximately the center of the leaf-blight lesion. Photographed from dried specimens collected near Los Baños, P. I., July, 1918. × ⅔

euchlaenae therefore can not be decided until additional information on the African form becomes available. It may be added that Zimmerman makes no mention of any leafspot on maize, although his account of maize rust makes it evident that this host came under observation.

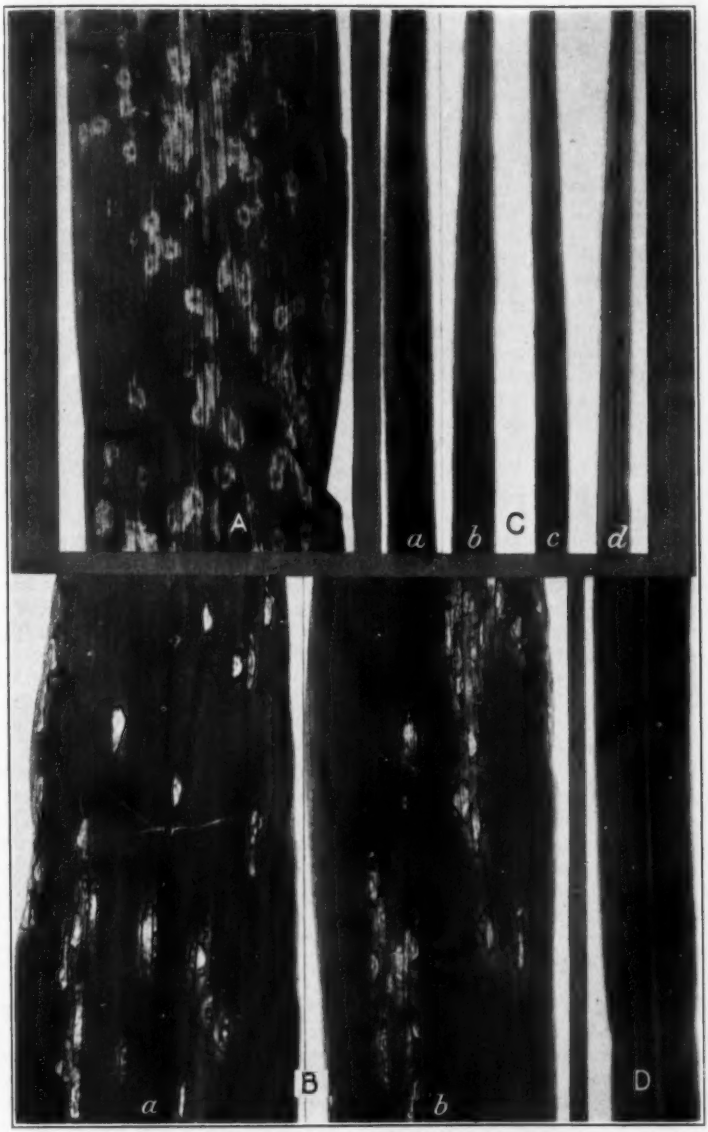
INOCULATIONS ON RICE AND SUGAR CANE

Morphologically, the parasite causing leafspot of maize shows much similarity to *Helminthosporium oryzae*, *H. sacchari* Butler, and *H. leersii* Atk. Perhaps its most obvious distinctive characteristic is found in the curvature of the conidium, this curvature being more pronounced than in any large-spored congeneric form known to the writer. The conidia of both *H. sacchari* and *H. leersii* would appear to be somewhat inferior in diameter to those of the maize parasite. Owing to a considerable degree of variability manifested by species of Helminthosporium under different conditions, it was believed advisable to determine the possible pathogenicity of the Florida organism on rice (*Oryza sativa* L.) and sugar cane (*Saccharum officinarum* L.), since these hosts are cultivated in tropical and subtropical regions where leafspot of maize is presumably of fairly widespread occurrence.

For the inoculation tests, plants grown in 5-inch pots in the greenhouse were used. The experimental material consisted of 24 pots each of sweet corn, dent corn, rice, and sugar cane, the maize being planted 3 seeds to a pot, the rice 10 seeds to a pot, and the cane started with a single cutting to each pot. Inoculations were begun 25 days after planting, when all the pots showed very satisfactory growth, and were repeated five times, at intervals of four days, the last material used thus being 45 days old, and in the case of the maize and cane, so large as to be somewhat unwieldy. Three pots of each host were inoculated at a time, by applying with an atomizer a suspension of conidia derived from pure cultures of the leafspot fungus on plates of corn-meal agar. Immediately after inoculation the plants, together with an unsprayed pot of each host as control, were incubated in a large moist chamber provided with glass windows.

On examining the maize plants 36 hours after inoculation, most of the leaves were found to be covered with hundreds of watersoaked spots varying in diameter between 1.0 and 2.5 mm., with the center occupied by a very minute whitish speck (pl. 2, A). The control plant immediately after this period showed no ill effects from confinement in moist atmosphere, but at the end of 48 hours showed symptoms of etiolation which became increasingly pronounced as incubation was continued to 72 or 96 hours. Even after 96 hours all except the lowest foliage of the controls was still functional, whereas the leaves of the inoculated plants had utterly collapsed and were converted into wet, softened structures drooping flabbily from the stalks.

An apparently more natural course of development of the infection resulted when the experimental plants were removed from the damp chamber to the greenhouse 24 or 36 hours after inoculation. Portions of the leaves in which the infections were very numerous withered very soon, to be sure, the healthy parts dying evidently from an interruption of the water supply, but where a more moderate



A.—Maize leaf 42 hours after inoculation by spraying with conidial suspension of *Ophiobolus heterostrophus*. Photographed by transmitted light, the light areas corresponding to watersoaked spots. × 3/2

Ba-Bb.—Portions of maize leaf inoculated with conidial suspension kept in damp chamber 36 hours, then in greenhouse 15 days. × 3/2

Ca-Cd.—Portions of rice leaf sprayed with conidial suspension and kept in damp chamber 42 hours, the incipient spots originally formed not subsequently increasing in size. × 3/2

D.—Portion of sugar-cane leaf sprayed with conidial suspension and kept in damp chamber 42 hours, the incipient spots originally formed not increasing in size. × 3/2

distribution prevailed the tissues withered only in the regions directly involved in the lesions. The enlargement of the latter took place especially toward the base and the tip of the leaf, lateral extension being in most instances, though not in all, checked by the veins. This elongation resulted in the characteristic lesions found on the original specimens—a striplike spot of uniform width, variegated with delicate, reddish-brown, elliptical or transverse zonation, and surrounded by a narrow reddish-brown marginal zone. The zonation, as expected, was found to have its origin in the intermittent extension of the lesion. During periods of high humidity prevailing often as a result of watering or the lowering of temperature during the night, a zone of healthy tissue immediately below and above the established lesion became watersoaked in appearance. With the recurrence of dry conditions, these portions of tissue withered and appeared as additional zonal increments (pl. 2, B, *a* to B, *b*).

In contrast to the heavy infection brought about on both field corn and sweet corn, most of the rice plants subjected to the same treatment showed no evidence of even incipient lesions. On two of the lots a sparse scattering of minute blackish specks appeared 36 hours after being inoculated. As the rice plants were found to flourish in the damp chambers, better indeed than in the greenhouse, the pots which had yielded the incipient infections were retained in the chamber for 20 days. The spots, however, increased neither in size nor in number, and at the time the material was discarded the foliage was in thriving condition (pl. 2, C, *a* to C, *d*).

The sugar cane responded to the inoculations in a manner somewhat similar to rice. Nearly all the cane plants developed minute, dark, rather indefinitely delimited specks on the foliage 36 hours after being sprayed with the suspension of conidia. As they appeared to suffer no ill effects from being exposed to the humid conditions of the damp chamber they were retained in the chamber for more than 20 days. None of the lesions, however, evidenced any tendency toward further development (pl. 2, D).

The vigorous parasitism of the fungus on maize foliage contrasts strongly with its failure to produce anything but incipient infection, in spite of especially favorable conditions for development, on the leaves of rice or sugar cane. That the organism would infect rice or sugar cane under natural conditions appears highly improbable. Its biological behavior is thus confirmatory of morphological evidence indicating its specific independence of *Helminthosporium oryzae* and *H. sacchari*.

ASCIGEROUS STAGE OF THE PARASITE

In discussing the specific identity of the fungus strains developing on the diseased maize leaves from Florida and from the Philippines, mention was made of their association with entirely similar ascigerous fructifications. The latter develop side by side with the conidiophores when diseased maize leaves are incubated in a damp chamber, although the minute black masses that constitute the young perithecia can not usually be discerned with the naked eye until the third or fourth day. Apparently the initial stages of development take place under the epidermis, which, however, is generally soon broken through and the sclerotiumlike bodies exposed to view. In its later stages

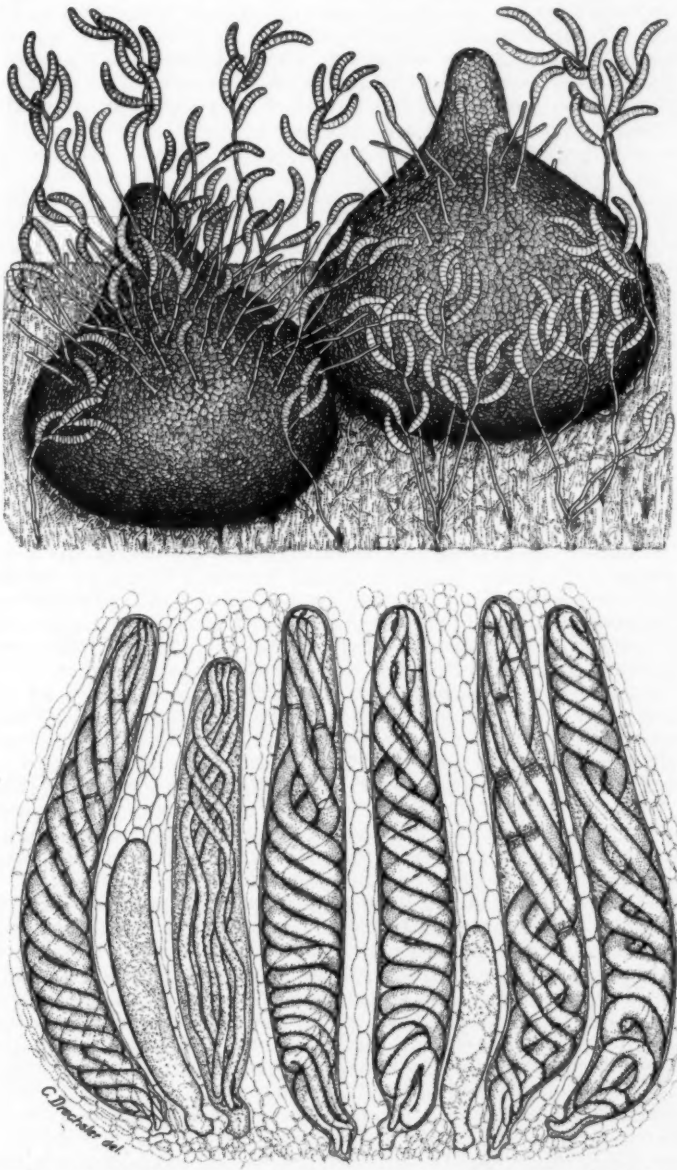


FIG. 4.—A (upper). Two mature perithecia of *Ophiobolus heterostrophus* on diseased maize leaves incubated 20 days in a damp chamber, illustrating some variation in size and shape, and relationship to conidial fructifications. × 120

B (lower). Asci in various stages of maturity together with vertically oriented filaments that compose the colorless pseudoparenchyma in the interior of the perithecium, which diminishes with the development of the asci. × 473

the fruiting body has the appearance of a superficial structure (fig. 4, A, upper). In some instances the rupturing of the disintegrating host tissues is delayed longer, while in the case of perithecia developing on the filter paper over which the fungus spreads from infected material, even the earlier stages are practically superficial, the loose mat of overlying fibers scarcely affording any substantial covering. The globose portion of the perithecium attains its definitive size in about 6 to 10 days, and a few additional days are usually required for the beak to reach its final proportions. Externally the fruiting body is black and relatively smooth, though often beset toward the base with stubby cylindrical projections resembling incipient conidiophores, or, perhaps, the proximal part of fractured conidiophores. The dark pseudoparenchymatous external layer is relatively thin, while the interior consists at first of firm colorless pseudoparenchyma composed of laterally appressed, vertically oriented filamentous elements. Young asci can generally be made out here and there after 12 days, and at the end of 15 days are not at all rare. As the asci increase in size and number the pseudoparenchymatous structures diminish correspondingly. Perithecia from 18 to 20 days old generally yield, on being crushed, a small proportion of approximately mature asci; the condition illustrated in Figure 4, B (lower), where most of the asci are mature, being, however, not attained usually in less than 25 or 30 days.

The mature asci, except for the short, markedly contracted stipe, are roughly cylindrical in shape, though generally widest about one-third of their length from the base, from which point the diminution in diameter toward the apex is more gradual than toward the base. When crushed out individually they frequently show more pronounced distention, evidently as a result of the imbibition of water preliminary to dehiscence, when such imbibition takes place in the absence of pressure normally provided by the surrounding asci and filamentous elements. Those in the center of the fruiting body are usually straight, while others near the periphery are distinctly curved, to conform with the curvature of the subspherical perithecial wall (fig. 5, A). Each ascus contains typically four cylindrical ascospores coiled in a close helix, each spore usually exhibiting approximately four turns, which alternate regularly with the turns of the other spores. As the lower ends of one or more spores thrust well into the short contracted stipe the helicoid arrangement at the base of the ascus is constrained into some irregularity. The upper ends of the spores similarly thrust into the apex of the ascus, yet, owing apparently to the more regular conformation of the apical portion, the departure from the spiral arrangement is usually very slight. In several hundred asci examined the rotation of the helix has been found to be always in the same direction; that is, on an upper focus with the ascus in a horizontal plane following the spore from the base toward the apex the coil appears at right and disappears at the left. The four spores might thus be compared to the threads of a quadruple left-handed screw.

The earlier stages in the development of the ascospores are not easy to make out, at least in unstained preparations, as the young structures are distinguished in the granular protoplasm only with difficulty. The spores first become clearly visible in the still growing ascus as filamentous bodies, approximately 3 μ in diameter and about

125 μ in length, consequently extending nearly the entire length of the ascus within which they follow an irregularly sinuous course. Subsequent growth ensues both in length and diameter, so that the final dimensions are frequently more than double those indicated. The length of the spores thus comes to exceed that of the ascus, a condition which is made possible by the helicoid coiling of the ascospores, the turns of which become increasingly close as maturation proceeds. After a rather tight arrangement has been effected, cross walls make their appearance, beginning at the upper end of the spore and proceeding downward.

When a perithecium containing mature asci is crushed in a water mount, a brisk discharge of ascospores ensues. As might be expected because of their involved arrangement, the spores of each ascus are discharged simultaneously, the discharge always taking place from the apex, and with enough violence to propel the spores a short distance through the water, the helicoid coils thereupon slowly becoming relaxed in loose, sinuous curves. Judging from the instances in which dehiscence is impeded, frequently as the result of immature condition, discharge is preceded by some swelling of the ascus (fig. 5, B, C, D), and circumscissile rupture in the apical portion of the ascus wall (fig. 5, E). Intertwined spores in groups of four have occasionally been observed adhering to the tip of the beak of perithecia examined in place, leaving little doubt that normally the spores in each ascus are discharged through the ostiole simultaneously as a group.

On being liberated, either naturally or by manipulation, the ascospores appear as filamentous structures distinctly fuliginous in coloration, tapering somewhat at the extremities (fig. 5, H. K). The constrictions at the septa which previously were not pronounced usually become more clearly accentuated, evidently as a result of some slight enlargement of the delimited segments. The contents are somewhat granular, especially immediately adjacent to the septa. In some instances a mucous envelope is apparent (fig. 5, L, M). When properly mounted in water the spores germinate very promptly by the production usually of one to eight germ tubes which may originate terminally from the tip or laterally from any part of the spore (fig. 5, F. G).

It should be mentioned that while the number of spores in an ascus is typically four, cases in which only three are present are by no means rare, and even one-spored and two-spored asci may be encountered occasionally. In the latter instances the asci are less completely occupied, the helicoid turns are looser and more irregular, although the direction of rotation remains the same. In cases of frustrated dehiscence, where the spores are only partly liberated from the ascus wall, germination is not materially impeded, the germ tubes arising from the lower segments of the ascospores perforating the surrounding membrane without any apparent difficulty.

Cultures made by placing fresh asci on corn-meal agar plates and transferring the resulting growth to sterile agar slants, show the same type of growth and produce the same type of conidiophores and conidia as are found on cultures originating from conidia. Conidia obtained from such cultures when applied to maize foliage have been found to give rise to lesions indistinguishable from the lesions resulting from the use of conidia from other sources. The facts regarding

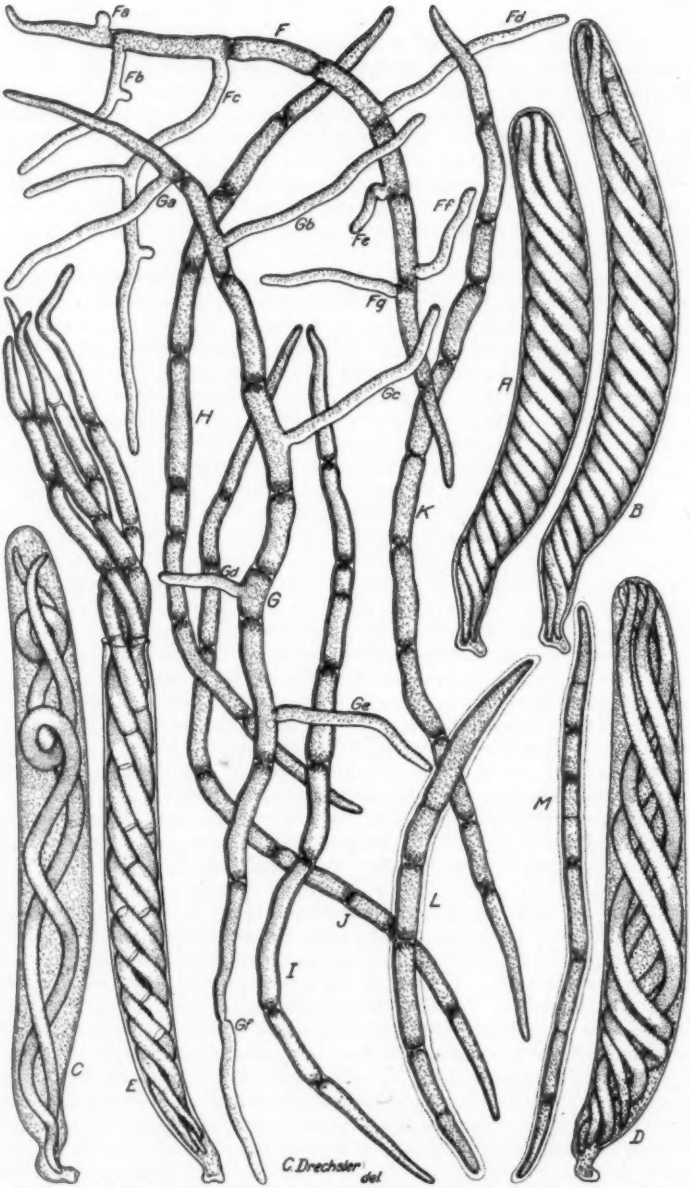


FIG. 5.—A. A mature or nearly mature ascus of *Ophiobolus heterostrophus* as obtained from a freshly crushed perithecium. × 476

B. The same ascus as in A, an hour later, showing elongation presumably preliminary to dehiscence; and the insertion of septa in the apical portion of the spores

C, D. Asci like B, after elongation, C containing only 2 spores. × 476

E. Partial liberation of ascospores by rupture of ascus toward apex; evidently an instance of frustrated discharge. × 476

F, G. Ascospores germinating by production of germ tubes Fa-g, Ga-f, respectively. × 476

H, I, J, K. Ascospores in mature septate condition. × 476

L. M. Mature ascospores inclosed in a mucous sheath. × 476

culture and pathogenicity only serve to confirm, however, the very clear evidence provided by the frequent presence on the perithecium of a larger or smaller number of conidiophores bearing conidia typical of the leafspot fungus and arising unmistakably from the perithecial wall (fig. 4, A). Sometimes the conidial fructifications arising in this manner become very abundant, with the usual result that the internal differentiation of the fruiting body is largely if not entirely suppressed.

CLASSIFICATION OF PARASITE

In reference to Saccardo's system, the fungus is presumably to be reckoned among the scolecosporous Sphaeriaceae, by virtue of the relative dimensions of the ascospores rather than because of any close similarity to the usual filamentous type. Among the definitions of the genera included in this category, none contain any special provision for the most noteworthy characteristic of the fungus—the disposition of the ascospore in a well-defined spiral. A considerable degree of similarity is discernible, however, to a fungus originally described by Saccardo (*12*) as *Rhaphidophora camptospora*, a combination later reduced by the author (*13, v. 2, p. 344*) to a synonym of *Ophiobolus camptosporus* Sacc. Under the latter binomial, Berlese published illustrations (*1, v. 2, pl. 159*) and a somewhat altered description (*1, v. 2, p. 133*) based upon material supplied by Saccardo. Berlese's figures show a short, stipitate, cylindrical ascus containing four spores partly coiled in helices in a manner strongly suggestive of the maize parasite, although the direction of rotation is opposite to that prevailing in the latter. In *Ophiobolus camptosporus* one of the median segments in the ascospores is swollen, which would seem to show relationship to certain other forms assigned to Ophiobolus, as well as to species referred to allied genera exhibiting one or more nodose intermediate segments.

Another fungus that should be mentioned in this connection is *Ophiobolus chaetophorus* (Crouan) Sacc., which, according to the account given by Malbranche and Niel (*6*), exhibits a spiral disposition of its ascospores suggesting the elaters of certain slime molds. In the figures, the direction of rotation seems to be like that found in the maize parasite. As the asci are described as eight-spored, and the perithecia as provided with rigid bristles on the upper part, differences from the latter organism are not lacking. *O. helicosporus* (B. & Br.) Sacc. (*13, v. 2, p. 350*) with "sporidiis linearibus praelongis spiraliter convolutis" and *O. galii* Rich. with "sporidiis spiraliter fasciculatis, hyalinis" represent two imperfectly described species that may possibly be related. Malbranche and Niel state that the perithecia of *O. helicosporus* are glabrous, thus differing in one particular from *O. chaetophorus*, which Saccardo later referred to the genus Ophiochaete, erected to include definitely setose forms.

According to recent publications by Höhnel (*4, 5*) and Weese (*16*), the genus Ophiobolus, as employed by Saccardo and other writers, is far from being a homogeneous group. Höhnel's recommendations contemplated the maintenance of three genera—(1) Leptospora Rabh., based on *L. porphyrogona* (Tode) Rabh. to include not only "sphaeriaceous" forms having narrow spores with or without a nodose cell, but also species having somewhat broader many-septate, colored spores with nodose segments; (2) Entodesmium Riess, based on *Entodesmium rude* Riess, a "dothidiaceous" type, to include those

species the spores of which become disarticulated within the ascus; and (3) Ophiobolus Riess, established on *O. acuminatus* (Sow.) Duby to include "sphaeriaceous" forms with nodose spores, presumably broader than those referable to Leptospora. An additional and new genus, Leptosporopsis, was proposed for certain other forms like *Ophiobolus rostrupii* Ferd. and Winge, *O. compressus* Rehm and *O. tanaceti* (Fuck.) Sacc. which Höhnel found to possess "dothidisceous" structure and hence to differ from Leptosphaeria de Not. only in having long narrow spores. Weese adopted Höhnel's revision of the group, suggesting, however, changing the definitions of the closely related "sphaeriaceous" genera Leptospora and Ophiobolus, so that the former is to include only forms with filamentous spores without nodose segments, while the latter is to include the species with nodose spores. For the setose forms, Weese retained two genera, Saccardo's Ophiochaete, to include forms with erumpent fruiting bodies analogous to Ophiobolus, and Acanthophiobolus Berlese to include species with superficial perithecia and worm-shaped, multiguttulate, subhyaline ascospores. Since the latter combination of characteristics was found represented in *Sphaeria chaetophorus* Crouan, the retention of the combination *Acanthophiobolus chaetophorus* (Crouan) Berlese, abandoned by its author in favor of Saccardo's combination, was indicated.

Although the taxonomy promoted by Höhnel and Weese may redistribute an assortment of fungi into a number of presumably less heterogeneous groups, it is not certain that the rearrangement proposed is, after all, as free of arbitrary features as might be desired. The distinction drawn between the "sphaeriaceous" and "dothidiaceous" types of discrete perithecia, on the basis of differences in internal development and structure, is one that has not hitherto been widely incorporated in mycological literature. In any case, the fungus under consideration would appear not to qualify for inclusion in Entodesmium as interpreted by Höhnel, as its spores show no tendency toward becoming disjointed. Nor could it well be referred to Leptospora, as the type for this genus, with its extremely slender spores, obviously does not represent a closely related form. While it must be admitted that the type of Ophiobolus, *O. acuminatus* (=*Leptosphaeriopsis acuminata* [Sow.] Berlese), with its asci containing eight straight spores, each provided with two swollen segments, does not itself show any close similarity, this species, from the information available, is at least not too obviously different from *O. camptosporus* with its ascus containing four helicoid ascospores each having one nodose intermediate cell to obviate the possibility of their being related more closely than by the arbitrary operations of analytical keys.

Judging from Berlese's figures of *Ophiobolus compressus* (*1, v. 2, pl. 156*) and *Ceuthocarpon brunellae* (Ell. and Ev.) Berl. (=*Leucospora brunellae* Ell. and Ev.) (*1, v. 2, pl. 170*), both of which Höhnel would place in his proposed genus Leptosporopsis, these fungi exhibit a degree of similarity to the maize parasite about equal to that shown by the general run of forms referred to Ophiobolus, though not as great a similarity as is evidenced by *O. camptosporus*. Indeed, neither Leptosporopsis Höhnel, nor Ophiomassaria Jaczewski, based on *O. selenospora* (Otth.) Jacz., a fungus with 2 to 3 septate ascospores in a mucus sheath (*13, v. 11, p. 353*); nor Acerbia as exempli-

fied in *A. therryana*, illustrated by Berlese (*1, v. 2, pl. 167*); nor Leptosporella Peng. and Sacc. (*13, v. 14, p. 619*), would seem to offer any species as similar to the maize parasite as *O. camptosporus*. In the absence of a more appropriate genus to which it could be referred, and in order to conserve for the time being its proximity to the latter form, the leafspot fungus is tentatively assigned to Ophiobolus. Such assignment is not intended to imply close relationship to certain species referred to this genus that are frequently mentioned in the literature pertaining to the diseases of cereal crops, as, notably, *O. cariceti* (Berk. and Br.) Sacc., and *O. herpotrichus* (Fr.) Sacc. It may be superfluous to add that the parasite is undoubtedly different from *Acerbia maydis* Rehm, described from dead remains of maize in the Philippines (*9*), as well as from *Leptosphaeria orthogramma* (B. and C.) Sacc., reported on dead maize culms in Pennsylvania, South Carolina (*13, v. 2, p. 60*), and the Philippines (*9*), for in respect to dimensions of spore, for example, the latter forms are decisively inferior to the fungus under consideration.

As far as the writer is aware, the ascigerous stage of the parasite has not been described before. There is scarcely any doubt that the conidial stage has been encountered by pathologists, but with the possible exception of the somewhat problematical *Helminthosporium euchlaenae*, it has apparently not served as type for any species designated by a binomial. As has been pointed out, Saccardo evidently did not intend to apply to it his binomial *H. curvulum*. The combination *H. turcicum* has long been applied, and seemingly altogether correctly, to the fungus causing leaf blight of maize and several other graminaceous hosts. The excessively brief diagnosis of *H. inconspicuum* applies better to the leaf-blight fungus than to the parasite causing leafspot, especially in the details concerning the 3 to 5 septate condition of the conidia and their diameter of 20 μ, although the passage "effused, but so thinly as not to be visible to the naked eye" is not readily reconciled with the actual appearance of *H. turcicum* developing luxuriantly on its host in nature. More direct evidence that *H. inconspicuum* is indeed synonymous with *H. turcicum* is provided by the specimen in the herbarium of the Office of Pathological Collections under the label:

> Ellis. North American Fungi. 45. *Helminthosporium inconspicuum* C. & E. Grev. vol. 6, p. 88. On leaves of Indian corn.

Which, judging from the date of issuance, 1878, presumably represents authentic material from approximately the same collection as the type specimens. The conidia present in abundance on the very extensive affected area were found to be altogether of the broad, straight, markedly tapering, relatively sparingly septate type characteristic of *H. turcicum*.

The specific name *heterostrophus*, descriptive of the ascospores, is suggested for the fungus.

DIAGNOSIS

Ophiobolus heterostrophus n. sp.

Occurring on maize (*Zea mays* L.), as the cause of a destructive disease manifested by the appearance on the leaves of numerous dead cinnamon-buff or purplish areas surrounded by a darker reddish brown margin, and often delicately variegated with brownish zonate bands; the lesions longitudinally elongated, first elliptical, later long-rectangular, typically limited to a single intervascular region, usually 1 to 3 by 5 to 15 mm., often coalescing to form more extensive dead portions.

Perithecia developing on disintegrating host tissues, usually early erumpent, black, often bearing a variable number of conidiophores, but no differentiated sterile setae; ascigerous portion subglobose or more frequently somewhat ellipsoidal, measuring usually 0.4 to 0.6 mm. in transverse diameter and approximately 0.4 mm. in vertical diameter; ostiolate beak well defined, subconical or paraboloid, approximately 0.15 mm. at base and 0.15 mm. in length; interior composed of colorless pseudoparenchymatous tissues consisting of vertically oriented appressed filaments, diminishing usually with the development of the asci.

Asci numerous, short-stipitate, with rounded apex, subcylindrical but sometimes becoming more inflated before discharge; 160 to 180 μ in length by 24 to 28 μ in diameter; containing 1 to 4, typically 4, spores. Ascospores filamentous, fuliginous, thin-walled; in somewhat immature condition, of uniform diameter of 6 to 7 μ, except at extremities which are somewhat attenuated; later becoming five to nine times septate, the septa usually associated with perceptible constrictions, the delimited segments becoming somewhat swollen so as to attain in places a diameter up to 9 μ; thrusting firmly against apex and into base of stipe in multiple heterostrophic helicoid arrangement with approximately four turns to each spore; measuring 130 to 340 μ in length; discharged simultaneously, often with mucous envelope; germinating promptly by producing indiscriminately from any or all segments, either laterally or terminally, germ tubes up to eight in number, from 3.5 to 5.0 μ in diameter.

Conidiophores arising singly or in groups of two or three from stomata in center of killed foliar parts; olivaceous, septate at intervals of 15 to 60 μ; bearing the first conidium after attaining a length of 50 μ or more; points of attachment of successive spores marked by scars occurring at intervals from 10 to 40 μ at geniculations not always pronounced; in nature measuring usually 120 to 170 μ in length, but under moist condition occurring as irregularly branching sporophoric filaments, often exceeding 1 mm. in length. Conidia developed at 25° C. on diseased maize leaves in damp chambers or in pure culture on artificial media, fuliginous to light olivaceous, measuring 30 to 115 μ in length by 10 to 17 μ in diameter; often strongly curved, usually widest near the middle and tapering perceptibly toward the rounded ends; peripheral wall thin, especially in the apical and basal regions; basal scar broad, not conspicuous, contained within rounded contour; germinating promptly by the production of two polar germ tubes.

Found on diseased leaves of *Zea mays* L. collected at Sanford, Fla., September 22, 1923 (type), Brooksville, Fla., June, 1917 (fig. 3, *Aa* to *Ae*), and at Los Baños, P. I., in 1918, 1919, 1920, and 1921. Perhaps identical with the more luxuriant Helminthosporium form widely occurring on the inflorescence of maize in tropical and subtropical regions. Found also on leaves of *Euchlaena mexicana* Schrad. near Los Baños, P. I., September and November, 1918; and possibly to be identified with *H. euchlaenae* Zimm.

TAXONOMIC CONSIDERATIONS

The pleomorphism of the maize parasite casts light on the affinities of a large proportion of the fungi referred to Helminthosporium. In their excellent account of *Pleospora polytricha* (*15, v. 2, p. 269–271, pl. 29*), the Tulasne brothers set forth an association with one representative of the genus which has since been paralleled by the discovery of analogous relationships in other graminicolous forms—*Pyrenophora teres* (Diedicke), *P. tritici-repentis* (Diedicke), and *P. bromi* (Diedicke) found in Europe and America, as well as *Pleospora graminea* Diedicke, the ascigerous stage of the parasite causing the widely distributed stripe disease of barley, which stage, however, has hitherto been reported only from Europe. A significant fact concerning all the species of Helminthosporium achieving their perfect form as members of the genera Prenophora or Pleospora is that their conidia are of the straight cylindrical type, germinating by the production of germ tubes laterally or obliquely from intermediate as well as end segments. In pure culture, on many artificial media, under ordinary laboratory conditions, these species are characterized usually by abundant anastomoses in the submerged portion of the mycelium, resulting in the production of complexes of inflated elements plausibly inter-

preted as incipient perithecia, and by conidial apparatus either being absent, or, if present, not infrequently represented by proliferous atypical structures in which sporophoric elements and spores are poorly differentiated.

The maize parasite, like *Helminthosporium sativum* P. B. and K., *H. sacchari* and *H. oryzae*—to mention only a few of the more important species—represents a type of Helminthosporium with mostly elliptical, curved conidia, germinating by the production of two germ tubes, both from small, thin-walled regions, one at the apex and the other immediately adjacent to and surrounding the basal scar. In artificial culture, sporulation is usually abundant and not markedly abnormal, although the conidia may be shorter and less regularly curved than those produced under natural conditions. There can be little doubt that the species adhering to this type, including many parasites of higher plants and very probably a considerable number of small-spored forms often referred to the conidial genus Brachysporium, are closely related with one another, and that the latter, when connected with ascigerous stages, be found not without similarity to *Ophiobolus heterostrophus*. It is apparent that a proper taxonomic disposition of the general run of species of Helminthosporium with bipolar germination is contingent on the discovery of the perfect condition of additional forms, from the morphology of which the common characters may be deduced for incorporation in the definition of a suitable genus, or, if possible, for appropriate revision of a genus already established.

SUMMARY

A foliar disease has been found to occur on maize in Florida and on maize and teosinte in the Philippines, which is characterized by cinnamon-buff lesions which are considerably smaller and much more numerous than those of leaf blight, and are also distinguished by being usually confined to a single intervascular region.

The disease is associated with a fungus which, in its conidial condition, differs from *Helminthosporium turcicum* in the smaller diameter of its conidiophores, as well as in the smaller diameter, more abundant septation, and greater curvature of its elliptical conidia, which, moreover, have an internal basal scar rather than a protruding modification.

The fungus produces discrete, subglobose perithecia with a well defined beak, and bearing asci containing typically four multiseptate, fuliginous, filamentous spores, each coiled in a heterostrophic helix of approximately four turns. It is described tentatively as *Ophiobolus heterostrophus*, n. sp., though not obviously closely related to several well-known species of Ophiobolus parasitic on grasses.

The morphological difference between this ascigerous stage and Pyrenophora or Pleospora lends support to the view that the species of Helminthosporium with straight subcylindrical conidia germinating laterally from end and intermediate segments constitute a natural group distinct from the group of species producing curved elliptical conidia germinating by two polar germ tubes.

The leafspot disease is probably widely distributed in tropical and subtropical maize-growing regions, having evidently been confused with leaf blight, which occurs in the same territory. It appears not improbable that one type of tassel mold will prove to be identical with the foliar parasite, in spite of the greater length of its conidia.

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THE TRANSLOCATION OF THE FOOD MATERIALS OF THE WHEAT SEEDLING [1]

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INTRODUCTION

At no time in the life history of the plant are the changes due to metabolism proportionally as great as in the seedling stage, when the reserve food of the kernel is changed into simpler and more soluble products and then used as a source of energy or as a part of the young plant. Many of the steps of anabolism and katabolism are poorly understood. Spoehr (*37*) gives a good summary of our present knowledge on this point.

It is the aim of this work to show the chemical and physical changes which take place during the germination of wheat.

Dehérain (*11*), Harz (*22*), Teller (*38*), and Willard and Swanson (*45*), have analyzed the materials found in the wheat kernel. Thatcher (*40*), Eckerson (*15*), and others (*25*, *39*, *2*), have followed the formation of these materials in the developing seed. Choate (*5*) has studied microchemically the changes in the carbohydrate and nitrogen content of seedlings during seven days germination in petri dishes. LeClerc and Breazeale (*28*), grew seedlings in various types of culture solutions for 15 days, and analyzed the seedlings for the mineral elements, ether extract, reducing and hydrolyzable sugars, pentosans, and fiber. Wimmer (*44*) studied the various parts of wheat plants, grown in soil, for minerals and starches at various periods, beginning at the time the plants were about 40 cm. high.

METHOD

Spring wheat of the Marquis variety, Clark and Martin (*6*), was grown in the greenhouse in earthenware containers, 17 by 19 cm., containing soil commonly designated "good field soil." The maximum water-holding capacity of the soil was approximately 50 per cent. The moisture content of the soil was determined and sufficient water added at the time of planting the seeds to bring the content to "optimum." The total weight was then recorded, and water was added frequently to maintain the optimum water content. The plants for the freezing-point determinations were grown in November and December, and those for the chemical determinations in January and February. The temperature of the greenhouse was about 55° F. at night and 70° F. during the day. The plants were collected in the morning before they were exposed to the sun's rays. Since the composition of the plants varies throughout the day and with the conditions varying on different days, as illustrated by the

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[3] Reference is made by number (italic) to "Literature cited," p. 741.

work of Davis, Daish, and Sawyer (*9*), and Davis and Sawyer (*10*), it was felt that these variations would be least in the morning. The adhering soil was carefully removed from the roots before they were washed. In order to prevent the drying out of the plants in transferring them from the greenhouse to the laboratory, they were placed in a desiccator which had been converted into a moist chamber.

For the freezing-point determinations, the plants were first dried of adhering moisture by blotting with filter paper, then ground in a mortar until they were reduced to a pulp. This was placed in cheesecloth and squeezed, either by twisting and pressing with pestle or in a tincture press. With various comparison, no difference in the depression was noted. Chandler (*4*), Dixon and Atkins (*13*), Knudson and Ginsburg (*26*), and Newton (*33*) have found it desirable to use high pressures in order to express the sap. This procedure seemed unnecessary, since in the first place the tissues at all times were young and hence not highly lignified, in which case it is always shown that the difference is small; in the second place, Knudson and Ginsburg's (*26*) work shows that when lower pressures are used to express the sap, the results more nearly coincide with the plasmolytic method. Salmon and Fleming (*35*), working with older wheat, found it necessary to treat the material with toluene or chloroform. The age of the plant also reduces very much the undercooling as discussed by Harvey (*21*).

Experiments were conducted to determine the effect of freezing this material before extracting the juice, as followed by Gortner and Hoffman (*17*). Comparisons were made with several sets of plants which were grown and treated in the same way in every respect, except that half of each portion was ground and extracted at once, while the other half was frozen for one and one-half hours before extracting. The material was placed in stoppered bottles. These were placed in quart thermos bottles containing a freezing mixture of salt and ice in the proportion which produces a temperature of 12°C. below zero.

TABLE I.—*Depression of the freezing point of sap from plants which were frozen and from those not frozen, and the difference in each case*

Date	Age of plants	Depression when—		Difference (+) or (−) when frozen	Date	Age of plants	Depression when—		Difference (+) or (−) when frozen
		Frozen	Not frozen				Frozen	Not frozen	
	Days					*Days*			
November 17	7	0.467	0.475	−0.008	November 6	13	0.407	0.390	+0.017
		.470	.480	−.010			.400	.390	+.010
Nov. 19	9	.360	.395	−.035	Nov. 13	15	.495	.470	+.025
		.360	.400	−.040			.480	.465	+.015
Nov. 5	9	.430	.415	+.015					
		.425	.405	+.020	Average difference				+.0045

The differences indicate that freezing has no consistent effect in increasing the osmotic pressure of the cell sap, and that the cells are not made more permeable by both freezing and grinding than by grinding only without freezing.

The 2-day-old seedlings formed a paste when ground, but not enough sap could be extracted from this paste to make the test.

Therefore the method as described by McCool and Millar (*31*) was followed in the determination of the freezing point of the ground tissue. The ground paste was placed in the freezing tube in such a way as to press gently around the bulb of the Beckman thermometer. The only precaution necessary to use in making the determinations in this way is to keep the freezing bath at about 4° C. Experiments on older plants indicate that the depression is slightly greater with the tissues than with the extracted juices, and this confirms the work of McCool and Millar (*31*). (Data are given in Table II.)

TABLE II.—*Depression of the freezing point of the juice and ground tissue of plants*

Age of plants	Depression in degrees		Increase in depression in ground tissue
	Juice	Ground tissue	
Days			
5	0.75	0.76	0.01
	.715	.765	.05
6	.61	.705	.095
	.62	.710	.090

Since the depression is somewhat greater for ground tissue than for extracted sap, the reading for the 2-day-old plants shows a depression slightly in excess of what we would expect.

In all cases except the 15-day stage with four samples, the results given are from the average of determinations of five sets of plants grown separately, and no more than two determinations were made in any one day.

Comparisons were made of the freezing-point depression of the sap of roots, plumules, and entire plants. The data are submitted in Table III.

TABLE III.—*Comparison of the freezing-point depression of the sap of roots, plumules, and entire plants*

Age of plants	Depression in degrees		Average	Entire plants
	Plumules	Roots		
Days				
10	0.545	0.260	0.4025	0.39
	.545	.250	.3975	.385
15	.600	.255	.4275	.43
	.575	.250	.4125	.43

The plumules have a depression nearly twice as great as that of the roots. If reference is made to Table IV it may be observed that the plumules have more soluble materials and less water than the roots. This would account for the difference in the freezing points. Averaging the depression of the roots and plumules, the depression is practically the same as for the entire plant. This is to be expected, as the green weights of the roots and tops differ by only a little.

TABLE IV.—*Carbohydrates, expressed as dextrose, and the nitrogen content of seeds, plumules, roots, and total plants expressed as milligrams, per 100 parts, per cent of dry weight, and per cent on the basis of the original amount in the seed*

Parts of plant and age	Weight in grams per 100 parts	Per cent of weight of original seed	Ether extract	Per cent of dry weight	Per cent of weight of ether extract of original seed	Reducing sugars	Per cent of dry weight	Total sugars	Per cent of dry weight	Per cent of weight of total sugar of original seed	Dextrin	Per cent of dry weight	Per cent of weight of original seed of dextrin	Starch	Per cent of dry weight	Per cent of weight of starch of original seed	Acid hydrolyzable	Per cent of dry weight	Per cent of weight of acid hydrolyzable of original seed	Nitrogen	Per cent of dry weight	Per cent of weight of nitrogen of original seed
Original seeds	2.685	.....	66.9	2.49	.....	.....	.....	53.86	2.00	.....	43.5	.....	.....	1,781.0	66.40	.....	249.5	9.30	99.0	59.07	2.20	.....
Plants:																						
1 day	2.708	100.80	63.1	2.52	94.2	.....	.....	44.7	1.50	83.0	82.0	3.03	198.1	1,621.3	59.91	91.10	246.9	9.12	99.0	54.41	2.01	92.20
2 days	2.593	96.82	57.6	2.30	86.0	22.76	0.91	137.15	5.29	255.0	111.0	4.28	255.3	1,079.0	41.60	60.59	243.5	9.39	97.6	46.20	1.78	78.22
3 days	2.5443	94.80	51.03	1.95	76.4	83.145	3.18	121.39	4.64	225.0	81.29	3.10	186.7	1,343.5	51.30	75.40	241.2	9.22	96.8	48.83	1.92	82.86
6 days	2.4761	92.20	45.95	1.85	66.7	234.775	9.48	465.80	18.80	864.2	120.51	4.88	277.2	472.1	19.05	26.50	259.3	10.47	103.8	49.64	2.00	84.02
9 days	2.3833	88.80	95.28	4.00	142.4	117.348	4.92	208.975	8.76	387.0	86.53	3.63	198.7	117.5	4.93	6.60	332.7	13.95	133.2	63.31	2.66	107.2
12 days	2.0307	75.70	94.71	4.46	141.5	15.59	.77	37.08	1.82	70.2	19.87	.978	45.7	20.2	.99	1.13	300.5	14.77	132.5	54.32	2.67	92.00
15 days	2.3554	87.70	122.76	5.25	183.5	5.45	.171	23.76	1.01	44.1	15.85	.673	36.4	14.65	.62	.82	295.4	12.54	118.5	79.84	2.38	134.90
25 days	5.173	192.50	310.20	6.00	464.0	10.06	.194	12.49	.24	23.2	13.57	.262	31.2	64.3	1.24	3.61	484.5	9.36	194.2	177.72	3.44	301.0
Seeds:																						
3 days	2.3317	86.9	46.3	1.99	69.2	76.50	3.28	105.90	4.54	196.0	75.35	3.23	173.1	1,339.5	57.4	75.12	217.2	9.31	87.2	37.56	1.61	63.60
6 days	1.8072	67.3	31.2	1.72	46.7	212.70	11.76	430.70	23.81	799.0	105.70	5.85	242.9	468.0	25.9	26.26	210.0	11.6	84.2	27.30	1.51	46.22
9 days	.8387	31.2	29.7	3.54	44.4	102.10	12.2	167.00	19.91	310.0	68.06	8.12	156.5	109.0	13.00	6.12	138.3	16.5	55.5	12.75	1.52	21.57
12 days	.2866	10.7	16.27	5.68	24.3	8.40	2.93	20.46	7.15	37.2	8.93	3.12	20.5	17.9	6.24	1.01	89.9	31.4	36.0	3.64	1.27	6.16
15 days	.279	10.4	13.65	4.89	20.4	1.25	.45	3.82	1.37	7.08	4.98	1.78	14.9	10.6	3.80	.60	88.8	31.8	35.6	2.18	.78	3.69
25 days	.250	9.3	12.85	5.14	19.2	0	0	.65	.26	1.20	2.92	1.18	6.8	5.9	2.36	.33	8.0	3.2	3.21	.93	.37	1.57
Plumule:																						
3 days	.1096	4.09	2.12	1.93	3.17	3.095	2.82	9.18	8.37	17.00	3.30	3.01	7.6	2.58	2.5	.14	8.91	8.12	3.57	5.88	5.36	9.95
6 days	.3832	14.3	8.65	2.26	12.92	13.585	3.54	20.42	5.33	37.90	9.58	2.50	22.0	2.86	7.47	.16	2.86	7.47	1.15	14.80	3.86	25.04
9 days	.8754	32.6	54.80	6.27	81.9	13.55	1.55	26.50	3.03	49.2	11.70	1.34	26.7	7.00	.80	.39	95.4	10.90	38.3	37.71	4.31	63.81
12 days	1.0536	39.2	68.25	6.48	102.0	0	0	4.09	.389	7.60	3.03	.288	7.0	0	0	0	77.8	7.38	31.2	34.80	3.30	58.95
15 days	1.4489	54.0	99.90	6.90	148.0	0	0	5.5	.38	10.2	4.77	.329	11.0	2.15	.148	.12	93.0	6.42	37.2	66.74	4.59	113.00
25 days	3.5730	133.2	272.50	7.63	407.5	0	0	0	0	0	0	0	0	21.5	.602	1.21	182.4	5.10	73.1	143.70	4.02	243.20
Roots:																						
3 days	.1030	3.84	2.6	2.52	3.89	3.55	3.45	6.30	6.12	11.68	2.46	2.39	5.6	1.45	1.41	.08	16.10	15.6	6.46	5.39	5.23	9.13
6 days	.2859	10.6	6.1	2.14	9.12	8.49	2.97	14.76	5.17	27.4	5.23	1.83	12.0	.61	.214	.03	46.48	16.25	18.61	7.54	2.64	12.75
9 days	.4692	17.5	10.7	2.28	16.0	11.73	2.50	15.47	3.30	28.70	6.77	1.44	15.6	1.47	.398	.08	98.97	21.05	39.65	12.95	2.76	21.94
12 days	.6905	25.7	10.2	1.48	15.2	7.19	1.04	12.53	1.81	23.40	7.83	1.13	18.0	2.27	.328	.12	132.75	19.21	53.21	15.88	2.30	26.89
15 days	.7275	27.1	9.2	1.26	13.7	4.20	.577	14.44	1.98	26.80	6.10	.841	14.0	1.90	.261	.11	113.55	15.62	45.51	10.92	1.50	18.47
25 days	1.3500	50.2	24.8	1.84	37.0	10.05	.744	11.835	.876	21.90	10.62	.786	24.4	36.85	2.73	2.06	294.10	21.80	117.8	33.09	2.45	56.00

The green weight, dry weight, and ash determinations, as shown in Table V and plotted in Figure 1, were made on plants grown as just described. The plants were counted, dried of excess moisture, placed in weighing bottles, then weighed to determine the green weight, and then dried rapidly in a vacuum oven at 80° C. to complete desiccation. The dried plants were then transferred to porcelain crucibles and reduced to constant weight for ash in an electric muffle furnace at dark-red heat. The ash determinations are expressed not only as grams of ash but also per cent ash of dry weight and per cent of weight of the original seed.

TABLE V.—*Green weight, dry weight, and weight of ash for 100 parts, expressed in grams, and per cent of ash in dry material, per cent of ash based on ash of original seed, and per cent of dry weight of original seed*

Age and plant	Part of green weight	Dry weight	Weight of ash per 100 parts	Per cent ash of dry weight	Per cent ash of original seed	Per cent dry weight of original seed
Original seed (air dry)	2.8990	2.6228	0.0534	2.03		
Plumule:						
3-day	.4760	.0896	.0059	6.58	11.0	3.42
6-day	3.8260	.3210	.0188	5.84	35.2	12.24
9-day	6.4740	.6401	.0534	7.22	100.0	24.40
12-day	8.7840	.8428	.0910	10.75	170.2	32.13
15-day	14.2085	1.3961	.1869	11.94	350.0	53.22
25-day	32.2880	3.2612	.3572	10.95	668.9	124.30
Roots:						
3-day	.851	.0819	.0063	7.73	11.8	3.12
6-day	3.860	.2460	.0229	9.31	42.89	9.37
9-day	6.578	.4521	.0465	10.27	87.08	17.23
12-day	9.520	.4889	.0628	12.85	117.61	18.64
15-day	12.3648	.5990	.0659	10.99	123.41	22.83
25-day	16.787	1.11850	.0976	8.25	182.77	45.18
Seed:						
3-day	4.284	2.3632	.0471	1.95	88.20	91.62
6-day	2.440	1.8072	.0316	1.74	59.18	68.90
9-day	3.066	.8694	.0141	1.63	26.40	33.15
12-day	1.995	.3384	.0095	2.79	17.79	12.52
15-day	2.014	.2622	.0062	2.36	11.61	9.99
25-day	1.308	.2432	.0088	3.60	16.48	9.26
Plant:						
3-day	5.611	2.5745	.0593	2.30	111.05	98.16
6-day	10.126	2.3742	.0733	3.09	137.27	90.52
9-day	16.118	1.9616	.1140	5.81	213.48	74.79
12-day	20.299	1.6701	.1633	9.77	305.60	63.71
15-day	28.5873	2.2513	.2590	11.50	485.02	85.83
25-day	50.383	4.6894	.4636	9.88	868.15	178.80

The plants used for the chemical analysis were separated into seeds, plumules, and roots. The material was dried in a vacuum oven at 80° C., which has been found by Link and Tottingham (*30*) to be a very successful way to treat carbohydrate material for the analysis of sugars. In cases where the sugar content was high, as for example in seeds from the 6 and 9-day-old seedlings, it was found necessary, even under these conditions, to use Petri dishes and have the seeds well separated instead of using the weighing bottles which were used in the other cases, in order to have the drying go on fast enough to prevent discoloration and acccompanying chemical changes. It was also necessary to avoid putting large quantities of material in the oven at one time (an amount which would dry enough in six hours to crumble nicely seemed to give the best results). In cases where it was dried more slowly it usually lost the bright, desirable color. This method is preferable to the alcohol method where small quantities

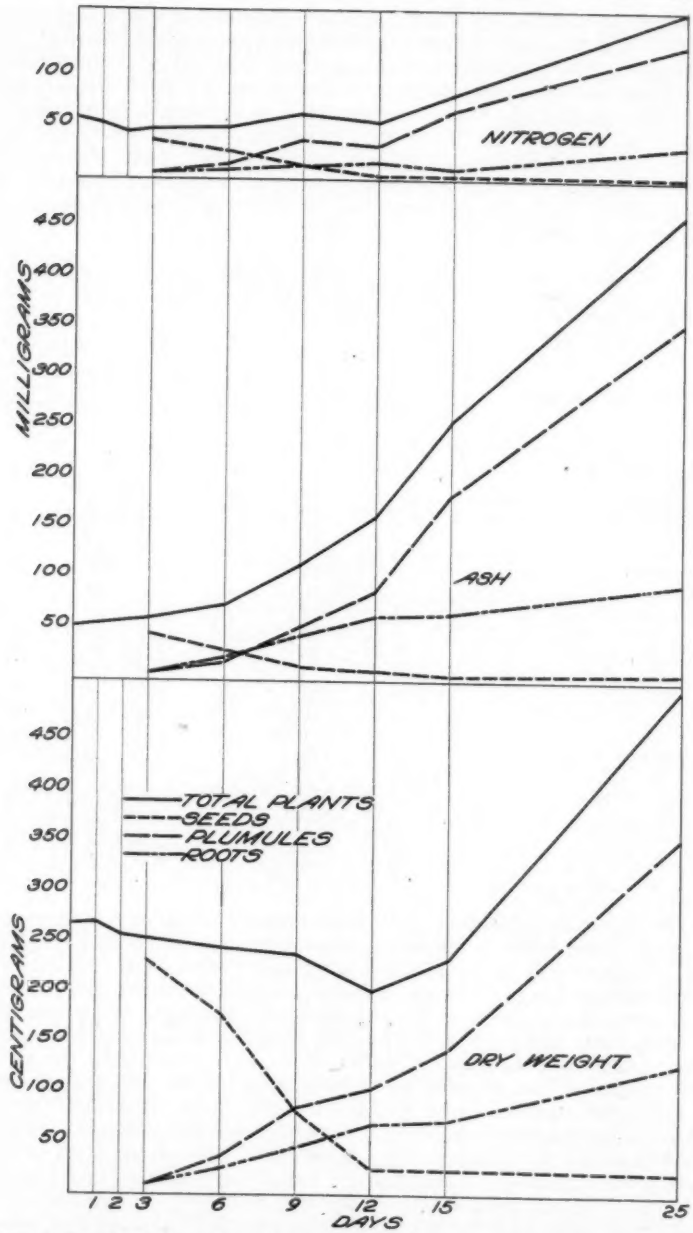

FIG. 1.—Dry weight expressed as centigrams, and ash and nitrogen expressed as milligrams. Solid line—total plants; short dash line—seeds; long dash line—plumules; long and short dash lines—roots

of material are used. Plants for each sample were collected on four or more different days in order to make a composite sample. The samples were ground in a pulverizing mill, often enough so that 90 per cent would pass through a 100-mesh sieve. The sample was then mixed and placed in bottles in a desiccator ready for analysis.

METHOD OF ANALYSIS

Duplicate samples which weighed between 1 and 1.5 gms. were extracted in a small Soxhlet extractor with anhydrous alcohol-free ether. This removed the lipoids and soluble pigments, which were determined by evaporating off the ether from the extract and weighing direct. The residue was placed in a 70° C. oven over night to drive off all the ether. The next day the residue was removed from the extraction shell to a 500 c. c. Erlenmeyer flask, and extracted in a water bath for one-half hour with 150 c. c. of 90 per cent boiling alcohol, to which a little calcium carbonate had been added to neutralize any plant acids which might be present. This was filtered and washed with 90 per cent alcohol. The extract was then reduced in volume under reduced pressure at 50° C., as described by Van Slyke (*42, p. 21*), to remove the alcohol. It was then diluted with water, clarified with lead acetate, filtered, freed from excess of lead by sodium carbonate, again filtered, and made up to 100 c. c. volume; 50 c. c. were used for the reducing sugar and 50 c. c. for the hydrolyzable sugars.

Reducing sugars were determined by the Munson-Walker method. The gas was regulated to bring the solution to boiling in the regulation time by using an oil manometer on the gas line. In this way it was possible to have the liquid begin boiling within 10 seconds of 4 minutes. The reducing sugars were expressed as dextrose from the Munson-Walker tables given by Leach (*27*).

Total sugars were determined by hydrolyzing the extracts with 25 per cent HCl.; 5 c. c. of HCl with 1.25 sp. gr. was added to 50 c. c. of extract, and boiled for an hour with a reflux condenser. This was neutralized with NaOH. The total sugars were determined by the Defren-O'Sullivan method, and expressed as dextrose.

In order to determine the dextrins, the residue from the sugar extraction was further extracted with 10 per cent alcohol at a temperature of 50° C. for one-half hour in a water bath. This was carried on in the same bath and while the sugars were being reduced in volume at 50° C. The filtered extract was concentrated for the removal of alcohol and clarified by the method just given. After hydrolysis with 25 per cent HCl for 1 hour and neutralizing with NaOH, the dextrin was determined as dextrose by the Defren-O'Sullivan method.

Starch was determined by boiling the residue from the dextrin extraction for 1 minute with 80 c. c. of water, in order to gelatinize the starch. After cooling to 38° C., the starch was digested with fresh saliva as suggested by Link and Tottingham (*30*), until a negative result for starch was obtained with iodine, then boiled and tested again with iodine; if negative, it was filtered, and the solution was hydrolyzed with 2.5 per cent HCl for 1½ hours, then neutralized with NaOH. The starch was then determined by the Defren-O'Sullivan method and expressed as dextrose. The fresh saliva

seemed more desirable than a commercial product used by Horton (*24*).

Hemicellulose, or acid hydrolyzable material, was determined by boiling the residue from the starch digestion with 100 c. c. of 2.5 per cent H_2SO_4 for 1 hour, with a reflux condenser. The solution was filtered and the filtrate neutralized with NaOH and clarified as above. The acid-hydrolyzable substance was determined by the Defren-O'Sullivan method and expressed as dextrose.

Total nitrogen was determined by using about 0.7 gm. of material from the composite samples. The Kjeldahl method as modified by Arnold-Gunning, with the further modification to include nitrates by the addition of salicylic acid, was used, and the results expressed as total nitrogen.

DISCUSSION

The dry weight of the total plants after germinating for one day is greater than the dry weight of the original seeds, but during the second and third day a considerable loss takes place. Doyer (*14*) points out that the chief changes in the first day of germination is the absorption of water, and little heat is given off. The greatest warmth is developed during the third and fourth days. Cribbs found by measuring the CO_2 given off during germination [4] that the respiration on the second day was about three times as great, and on the third day about five times as great as the first day. This small amount of respiration and loss of weight, with the gain in weight due to the hydrolysis of starch, may account for the slight increase in weight. The seed has the most rapid loss in weight between six and nine days, which is a period of rapid growth, before the plant has developed far enough to make carbohydrates rapidly. It is, however, increasing its ash content. The lowest dry weight is reached at 12 days, after which the anabolism is more rapid than the katabolism. The dry weight of the plumules seems to be always greater than the dry weight of the roots.

The use of storage fat by the seed has been studied extensively on fatty seeds. A comprehensive history is given by Miller (*32*). Waśniewoski (*43*) reports an increase in the amount of fat during the germination of wheat seedlings and suggests that some is formed from starch. This is in disagreement with most of the earlier work and with results reported here, which do show, however, that the fats of the seed are used more slowly than the other reserve foods in the seed. Spoehr (*37*) says "fats are important, especially in the development of seedlings." The other extract decreases slowly in the total plants up to six days, but at nine days a decided increase, particularly in the plumules, takes place. This is probably due to the chlorophyll. By referring to Table IV or Figure 2, it will be noted that at nine days the plants have about established the normal amount of ether extract, since the percentage in the plumules and roots remains about constant from this point on. The roots have a higher percentage of fat in the young stages than in older stages, which suggests that some of the fat may be translocated as fat and is later used by the root.

[4] CRIBBS, W. J. THE EFFECT OF FREEZING SEEDS ON THE RESPIRATION AND CATALASE ACTIVITY OF SEEDLINGS. 1923. [Unpublished thesis, Univ. Chicago.]

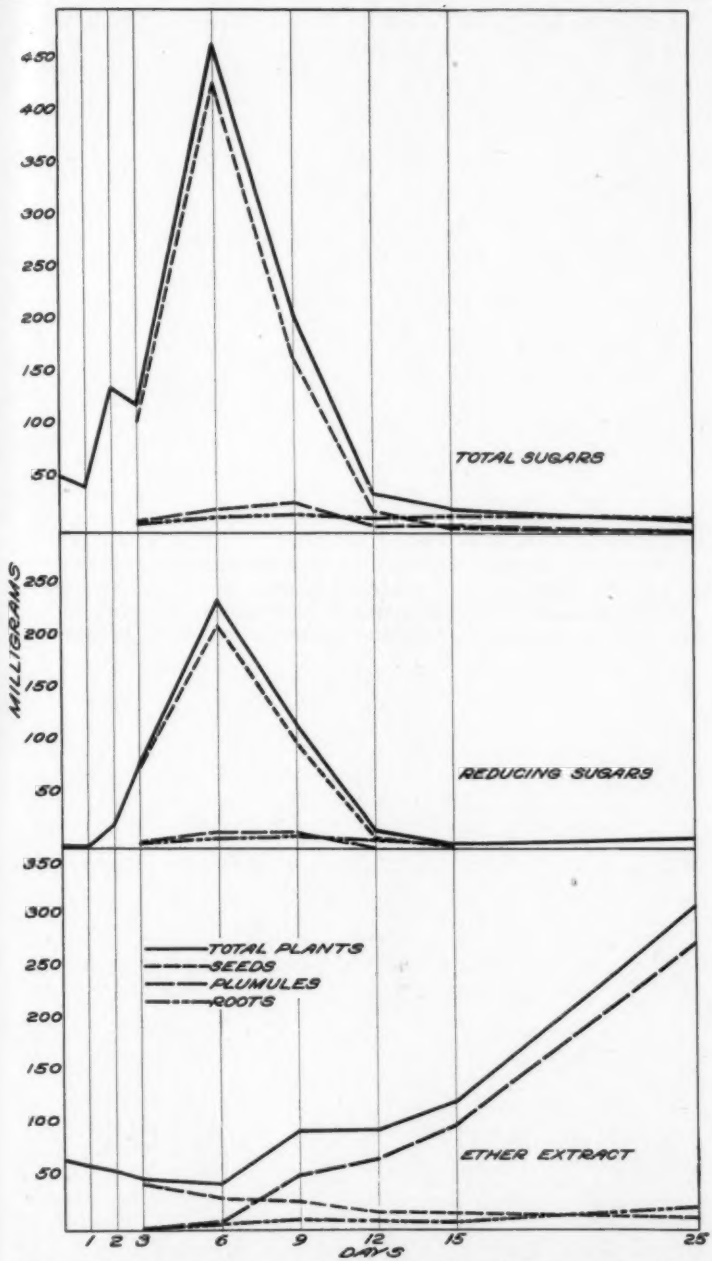

FIG. 2.—Ether extract, reducing sugar, and total sugars, expressed in milligrams. Solid line—total plants; short dash line—seeds long dash line—plumules; long and short dashes—roots

The seed contains no reducing sugar. Choate (*5*), by microchemical means, was able to detect reducing sugars at 18 hours. Under the conditions in which this material was grown, no reducing sugar was found until the second day. At two days there is good reason to believe the sugar to be maltose rather than a hexose sugar. In fact at no time is there evidence of more than a very small amount of hexose sugar. Le Clerc and Breazeale (*28*) found the amount of reducing sugars in the axes increased up to about the ninth day, when 100 axes (plumules and roots) contained 262.3 milligrams of reducing sugar. The greatest amount of reducing sugar shown by this work is also at the ninth day, but the amount is only 25.3 milligrams in 100 axes. The reducing sugar in the seed in the two cases parallel each other remarkably well, both in the maximum amount and in the increase and decrease. The source of the difference in these results must, therefore, lie first in the rate at which the starch is hydrolyzed in the seed, being more rapid in the case of plants grown in water cultures than in the case of plants grown in soil. The excess sugar formed in water cultures must then be translocated to the axes more rapidly than it is required by those organs, while, when grown in soil the translocation is proportional to the rate it is used. Le Clerc and Breazeale (*28*) have shown nearly equal percentages of sugars in the axes and in the seeds, while these results show 12 per cent of sugar in the seeds and less than 2 per cent in the axes, in 9-day-old plants. Green (*18*) found invert sugar to increase during 13 days germination of castor-oil beans. Miller (*32*) reached the same conclusion with *Helianthus annuus* seeds. Newton (*33*) reports larger quantities of reducing sugars in leaves of hardened winter wheat.

The data here presented as total sugar show no evidence of sucrose formation taking place in the seed. The plumules appear to have sucrose in addition to sugars of translocation. Brown and Morris (*3*), working with barley, were unable to find that maltose passes from the endosperm to the embryo, but assumed that it changed to simple sugar during translocation. When a larger amount of sugar is found after hydrolysis than before, it is assumed that sucrose is present. Le Clerc and Breazeale (*28*) did not determine the latter point, but they agree that no sucrose is formed in the seed. Colin and Belval (*7*) find sucrose to increase regularly in the stem. Translocation has taken place more rapidly into the roots than the sugar is used. Newton (*33*) finds as much as 16 per cent of the dry weight of winter-hardened winter wheat leaves to be sucrose. Green (*18*), in castor bean, finds first a decrease and then an increase of sucrose up to 13 days. Choate (*5*) finds no maltose in the seed.

The dextrins follow very closely between the starches and sugars, of which they are intermediate products. Where the starch shows a rapid loss the dextrins increase, and they drop off at about the same rate as the total sugars. At no time is there enough dextrin in the axes to indicate that translocation of dextrins takes place. Lindet (*29*), working with germinating barley, reports no dextrin, but suggests that the starch is changed to sucrose directly.

Starch is the principal storage polysaccharide of wheat, although a certain amount of triticin is reported by Thatcher (*41*). Spoehr (*37*) points out that it is not the total amount of carbohydrates, but the

degree of chemical inversion that determines the supply of material available for respiration and growth. Waśniewski (*43*) reports a constant relation between the starch decomposed and that consumed in respiration, regardless of the temperature, light, and changes in nitrogenous and inorganic substances. Doyer (*14*) says "a great loss of energy takes place the first seven days, mostly the third and fourth days." In the work here presented, the graph of Figure 3, showing the starch content, drops rapidly the first seven days, but the decline is extreme from the third to the sixth days. The sugars are highest at six days. The proportional increase in the dry weight of the axes was greatest at this period. All these facts substantiate the conclusions of the above investigators, showing that the greatest starch hydrolysis and greatest sugar content accompany greatest growth.

Small amounts of starch are found in the axes at all stages here examined, but with a slight increase at the 25-day stage. Newton (*33*) found no starch in the leaves of winter wheat when hardened, Wimmer (*44*) found starch in all parts of the wheat plant which were about 6 weeks old—about 2 per cent in the leaves, 6 per cent in the stems, 2 per cent in the roots, and an average for the whole plant of 3.5 per cent. Choate (*5*) found no starch in the plumule or the root; however, her seedlings were kept in the dark. In considering the evidence just given, it would appear that if the seedling has good growing conditions it will store starch in all the tissues, but if conditions are not favorable no starch will be found.

The acid hydrolyzable or hemicellulose content of wheat seedlings is made up largely, according to the classification given by Sherman (*36*) of xylem, found in the seeds and the tissues of plumules and roots. The percentage decreases slightly in the first three days, due to the action of cytase on the cell walls of the endosperm; after this the percentage based on dry weight increases, denoting that the hemicellulose is largely in the seed coverings which are not used. The percentage of hemicellulose remains about constant in plumules and in roots, but is twice as great in the roots as in the plumules. The total amount of acid-hydrolyzable substances expressed as dextrose increases from 3 per cent.

The nitrogen is not converted into protein by multiplying by a factor, since it is felt that, while it would be desirable to express the nitrogen of the seed as protein, which is described by Osborne (*34*), it would be incorrect to use the same factor for the nitrogen of the roots, which are high in nitrogen of other forms. Deleano (*12*) has shown, with leaves of *Vitis vinifera*, that as long as carbohydrates are present the protein is not used in respiration. Davidson and Le Clerc (*8*) find that the amount of nitrogen in the plant depends somewhat on the soil; when nitrogen is low in the soil the plants are usually lower in nitrogen. Gericke (*16*) has found a similar relation.

Table IV and Figures 1 and 3 show that the seed loses the nitrogen more slowly than the starch. The protein remains nearly constant in percentage of dry weight until the ninth day, which is the age at which the carbohydrates become deficient. Choate (*5*) found that the proteins of the endosperm are the first to be used, while the protein of the aleurone layer remains until very late in the germination stages. A large amount of nitrogen is translocated, as shown by the

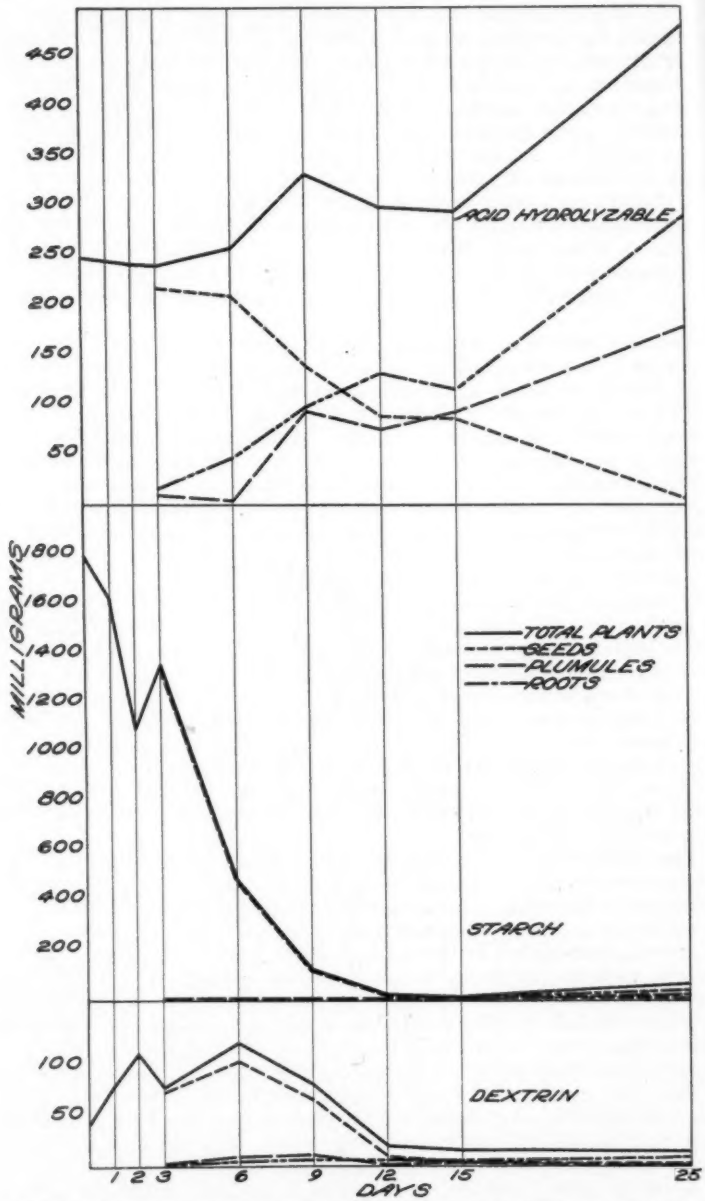

FIG. 3.—Dextrin, starch, and acid-hydrolyzable substances, expressed as milligrams. Solid line—total plants; short dash line—seeds; long dash line—plumules; long and short dashes—roots

high percentage of that element in 3-day roots and plumules with proportional loss in the seed. At 6 days the total plant has almost the same percentage of nitrogen as the original seeds and after that the percentage is always higher.

Haigh (*19*) concludes that wheat takes up nitrogen very rapidly in the seedling stage. Hoagland (*23*) has obtained results indicating that barley acts in the same way with respect to nitrogen during the early growing stages.

The ash of wheat seedlings changes more rapidly, and the maximum increase is greater, than the nitrogen or the carbohydrate content. The ash of the plumules and roots expressed as per cent increase over that in the seed is conspicuous, due to its rate of increase. A 3-day-old root with a dry weight of less than a milligram, which is 3 per cent of the original seed, is nearly 12 per c ent ash. Thus the minerals are deposited in the roots more than fourtimes as rapidly as the other substances. Plumules of the same age are a little heavier and contain 11 per cent ash. The close agreement in the amount of ash in the roots and plumules indicates that the minerals which are absorbed from the soil by the roots are translocated at about the rate that they are taken up. The total ash of 3-day-old plants was 11 per cent greater than that of the original seeds. The leaching from the seeds to the soil, if such a loss take place in soil as is described by Le Clerc and Breazeale (*28*) in water cultures, must be recovered by the plant at three days, besides the total increase which is found, hence the total intake of minerals is exceedingly large for this early development. The ash is not used from the seed as rapidly as the organic material, and the per cent of ash in the seeds falls slightly for nine days, then increases. Nine-day-old plumules have as much ash, and the 9-day root nearly as much ash, as the original seed, while the dry weight is less than one-fourth as great. The percentage of ash in the plumules continues to increase up to 15 days, when it is nearly 12 per cent, while in the roots the maximum percentage is attained at 9 days. Haigh's (*19*) work indicates a rapid intake of nutrients in the young wheat seedling. While Wimmer (*44*) found in the first crop of plants—that is, those a little larger than the ones used here—8 per cent ash; and when ripe the content was 2.33 per cent. The apparent small increase in the ash of 25-day-old seeds may be due to the fact that at this time much infection of seed has taken place and the lower organisms may be adding ash.

The rapid intake of nitrogen and the mineral elements by the young wheat seedling indicates that the plant is able to utilize the largest amount of these elements during the first few weeks of growth, and that the mineral supply is the limiting factor for the development in these young stages. Minerals, Figure 1, are the only substances which actually increased as early as the third day. It may be mentioned that in common agricultural practice fertilizers with a relatively high amount of nitrogen are applied to soils low in fertility in order to give the wheat a more rapid early growth. Davidson and LeClerc (*8*) found that early applications of nitrogen increased the yield. Osmotic pressures are given according to the work of Harris and Gortner (*20*).

TABLE VI.—*Concentration of the expressed cell sap*

Age of plants (days)	Length in centimeters		Dry weight in grams	Freezing point of expressed juice, depression in °C.	Osmotic pressure in atmospheres
	Tops	Roots			
2	0.15	0.7	0.0257	0.8910	10.73
3	.50	1.5	.0247	.8135	9.795
4	1.40	5.2	.0244	.676	8.021
5	3.00	6.3	.0225	.646	7.76
6	4.7	8.0	.0196	.5877	7.08
7	6.5	10.6	.0199	.552	6.65
8	8.5	14.4	.0178	.4995	6.02
9	9.4	15.6	.01939	.4555	5.367
10	11.5	16.3	.01905	.4205	5.068
11	11.7	16.3	.0193	.4170	5.026
12	12.2	18.5	.0201	.4245	5.116
13	13.2	20.0	.0195	.4030	4.857
14	13.6	19.5	.0210	.4275	5.272
15	16.4	20.9	.0209	.445	5.362
25	19.5	27.8	.0396	.497	5.99

If one follows in Table VI or Figure 4 the depression of the freezing point of the cell sap of wheat seedlings, it will be noticed that there is a

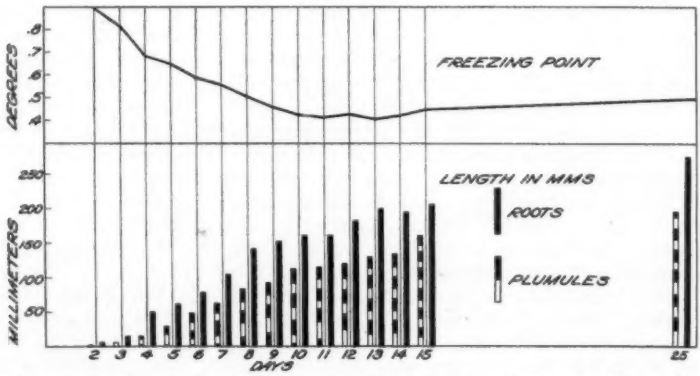

FIG. 4.—Length of roots and plumules, in millimeters; and freezing-point depression of the cell sap, in degrees

gradual decrease from the 2-day-old plants to about the 12-day-old plant stage, after which the osmotic pressure becomes greater. The depression of the freezing point is obviously due to the factors already discussed—namely, the amount of water and soluble substances. The amount of moisture becomes greater after the 2-day stage, which tends to decrease the osmotic pressure. The rôles played by the sugar and ash elements reach their maximum values at different stages. In the 3-day stage the ash is very high, being nearly 2 per cent of the moisture content; the sugar is lower at the 3-day stage than at the 6-day stage. The percentage of ash, based on moisture content, drops from the 2-day-old stage to the 9-day-old stage, from which point there is a continual increase. The sugars gradually but continuously decline in percentage. Bakke's (*1*) work indicates by the rate of transpiration that the plant contains more water in the morn-

ing. Yuncker (*46*) found the maximum photosynthesis took place in the corn plant about noon. These two factors would tend to increase the depression of the freezing point of stages effected by transpiration and photosynthesis if the plants were collected during the middle of the day and bring about less difference in the 2-day-old stage and later stages. These conditions could not be controlled from day to day; therefore it was thought advisable to avoid that period. The morning collections of material obviously give more accurate results on the plant's composition.

SUMMARY

During the early stages of the germination of the wheat seedling, the ether extract disappears from the seed more slowly than the carbohydrates or nitrogen, and accumulates in the plumules and roots more slowly than any other food material investigated.

The small amount of sugars normally found in the wheat kernel increases rapidly in the seed during the first six days of germination when nearly three-fourths of the starch has been used. After six days, the amount of sugar decreases. The sugars are translocated to the plumules and roots more rapidly than they can be used, while there is a supply of starch in the seed, but after nine days there remains approximately 1 per cent of the original starch; the sugars in the axes decrease sharply. These plants are dependent upon their photosynthetic powers for carbohydrates after 12 days. Under the conditions of this experiment, the embryo seemed to depend on the endosperm for about six days for its carbohydrate supply. During the period between 6 and 12 days the plant seems to become independent.

The hemicelluloses appear to be of little importance to the developing plant. The plumules and radicles show no marked difference in the percentage of acid hydrolyzable materials during the germination period.

Nitrogen is translocated rapidly in the three-day seedling, but after that the percentage remains constant in the axes. The nitrogen content of the seed decreases in proportion to the weight the first nine days; after that a more rapid loss occurs.

Wheat seedlings require proportionally larger quantities of minerals than any other food material. The maximum ash content of plumules and radicles is reached in 12 to 15 days. The roots appear to take in minerals more rapidly than they are translocated in the early stages of germination.

The concentration of the cell sap decreases from 2 days to 12 days, after which an increase takes place. This follows closely the changes in water, sugar, and ash contents of the plant. No elaborate method of extracting the sap was found necessary.

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POSSIBILITIES AND LIMITATIONS OF CHLOROPICRIN AS A FUMIGANT FOR CEREAL PRODUCTS[1]

By R. N. CHAPMAN, *Associate Professor of Entomology*, and A. H. JOHNSON, *Strietmann Fellow in Agricultural Biochemistry, Minnesota Agricultural Experiment Station*[2]

INTRODUCTION

There has been a demand for a substitute for carbon disulphide for fumigating cereals and other stored food products to kill the insects which infest them. Carbon disulphide had many satisfactory properties, but its inflammability led insurance underwriters to adopt a ruling which renders an insurance policy void if carbon disulphide is used or stored on the premises of an insured building, and, likewise, practically all railroads have ruled against the use of it in their freight cars.

The great amount of research work which was done on poisonous gases during the war has been drawn upon by workers in nearly every country, but it has not been easy to find a material sufficiently toxic to insects which would not at the same time be injurious to the materials to be fumigated.

This paper is devoted to the results of a study of the toxicity of chloropicrin to certain insects which infest cereals, and to the effect of the chloropicrin on the cereals and their products.

The chloropicrin used in the experimental work was obtained from the Edgewood Arsenal. It had a specific gravity of 1.6595, compared with water at 20° C. Bertrand (*3*)[3] states that chloropicrin prepared by the action of calcium chloride on picric acid has the following properties: Density at 16° C., 1.666; boiling point at 766 mm. pressure, 112° C.; vapor tension at 15° C., 30.2 mm.; non-inflammable; 1.65 gms. soluble in a liter of water at 18° C.

TOXICITY TO INSECTS

SUMMARY OF LITERATURE

The toxicity of chloropicrin to insects was noticed by Moore in 1917 (*15*) while he was making a comparison of the volatility of organic compounds with their toxicity to insects. His table showed that, on the basis of the amount required to kill flies in 400 minutes, a gram molecule of chloropicrin was about 283 times as toxic as a gram molecule of carbon disulphide. He suggested that its toxicity was very likely due to its action as an enzyme poison.

A second paper by Moore in 1918 (*16*) gave the results of some experiments to determine the effects of chloropicrin on some of the insects which infest stored food products, on some of the products themselves, on the germination of seeds, and also on certain fabrics which are used for clothing and other purposes. The experiments were performed, for the most part, in wooden boxes, and the report shows

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[3] Reference is made by number (italics) to "Literature cited," p. 759.

only the amount of material required to kill in 24 hours. Moore concluded that one-half pound of chloropicrin per 1,000 cubic feet was sufficient to kill most insects, and that 1 pound would give satisfactory results with all those tried.

Since 1918 many papers have been published on this subject. In general, they are devoted to work on the various phases of toxicity on one hand and to use of the fumigant under practical conditions on the other. Notable among the papers of the first group are those by Bertrand and his associates in 1919, 1920, and 1921 (*3–9*). Those writers showed that as regards the toxicity of chloropicrin to insects, time and concentration bear an inverse ratio to each other. Bertrand also called attention to the fact that there was greater variation due to temperature than would be expected on the principle of Van t'Hoff and Arrhénius. He believed this to be due to the fact that much of the chloropicrin was retained in the tissues and continued to act after the insects had been removed from the gas, thus rendering the observation inaccurate so far as the time factor was concerned. It is interesting to note that when his results for toxicity with a concentration of 20 grams to the cubic meter are plotted against time and temperature, the results fall along a straight line continuing down to 0° C. This is noteworthy when one considers that nearly all other fumigants have their toxicity so reduced at 12° or 15° C. that they are of little practical value at these temperatures.

The results of other investigators are in accord with these of Bertrand and his associates, with regard to the toxicity of chloropicrin, and there is agreement that the effect of the chloropicrin is probably due to action on enzymes. Remy (*18*) described paralysis of certain individuals of *Argas reflexus* which survived fumigation; and Spencer (*19*) believed that the action was cumulative, resulting in the death of nearly all of the insects which at first gave evidence of having survived the fumigation.

Regarding the practical use of chloropicrin we have the results of Burkhardt (*10*), Feytaud (*13*), Remy (*18*), Spencer (*19*), Wille (*20, 21*), Delassus (*12*), and others. Here we do not have the general agreement as to its promise that we have with regard to the laboratory experiments on its toxicity. Delassus (*12*) and others advocate greater use of chloropicrin, and express the belief that the technique of using it needs improving. Burkhardt (*10*) reports poor results under conditions similar to those which gave good results with carbon disulphide and concludes that chloropicrin might be suitable for use in small receptacles which could be tightly sealed, but that it is not suitable for use on a large scale.

TOXICITY EXPERIMENTS

In the present writers' work, the first series of experiments was performed to determine the toxicity of chloropicrin under laboratory conditions. The chloropicrin was measured out in a small calibrated pipette and placed in a small stoppered vial until the insects had been introduced into glass jars of 8-liter capacity. The insects were placed in small bolting-cloth bags and suspended in the jars by means of threads, the ends of which hung over the edge of the mouth of the jars. The edges of the jars were greased with vaseline in order that there might be no leakage about the threads when the glass cover was in place.

Ten insects were placed in each sack and the sacks were suspended in the jars. The stopper was then removed from the small vial containing the dose which had previously been measured out, and the chloropicrin was carefully sprinkled over the bottom of the jar. The vial was then dropped to the bottom of the jar and the glass cover put in place.

The insects were watched carefully and the first sack of insects was withdrawn as soon as all movement had ceased. The remaining sacks were withdrawn in accordance with a schedule which was arranged on the basis of the time required for the movement to stop. As soon as a sack was withdrawn it was placed in the open air and left for a period of 48 hours, at the end of which time the per cent of dead insects in each sack was noted. As a rule, at least some of the insects revived in the sack which was first removed from the jar. Each successive sack usually had fewer survivors, until finally there were none. The time of exposure of the first sack in which there were no survivors was taken as the time required for 100 per cent kill for the concentration used in the experiment. In general, the decrease in the number of survivors was much more regular in the successive sacks when a high concentration was used than with a low concentration. The controls were left in sacks in the laboratory and were counted with the beetles from the experiments.

The variation found when using some of the lower concentrations was often very confusing, and this may help to explain some of the discrepancies met with in the practical application of chloropicrin. In one experiment there was a difference of more than 15 per cent between two different sacks in the time required to kill 100 per cent. Since no such irregularities were observed in the controls, these differences may have been due to certain sacks becoming wet with the chloropicrin when the dose was introduced, or to some of the insects closing their spiracles for a time when first put into the jar.

The granary weevil, *Calendra granaria* L., was used in the experiments reported in this paper. Bertrand (*3*) used the rice weevil *Calendra oryza* L., but this species was not available during the present work, so the granary weevil, being very closely related and therefore assumed to be comparable in susceptibility to chloropicrin, was chosen. The results are plotted in Figure 1. Time is recorded in minutes, and concentration in grams per cubic meter. The crosses represent the records of Bertrand (*3*), and the dots the results of the present work. Bertrand (*5*) states that the temperature varied between 20° and 27° C. during his experiments, while that in this present work varied between 22° and 24° C. The results are in general accord when it is considered that Bertrand had a variation of 7° in the temperature under which he worked. The inverse relationship between time and concentration seems to hold from the lowest to the highest concentration.

Figure 2 shows the relationship of the time and temperature factors at a concentration of 20 grams per cubic meter. Again the dots represent the results of the present experiments, and the crosses those of Bertrand (*6*). It is clear that time, temperature, and concentration are important factors. A low value for any one may be compensated for by giving a correspondingly high value to one or more of the others. No relationship was found to exist between relative humidity and toxicity.

The susceptibility of various species of insects to chloropicrin is different. The results of Moore (*16*), Bertrand (*5*), and others agree with those of this present work in showing that the confused flour beetle, *Tribolium confusum* (Duval) is more resistant than the weevils worked with, from 30 to 50 per cent greater dosage being required to kill it.

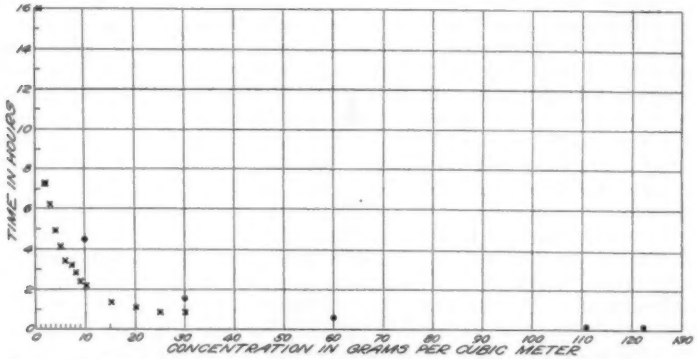

FIG. 1.—Time and temperature curves for toxicity of chloropicrin to *Calendra granaria* L. (used by these writers in this work) and *Calendra oryza* L. (used by Bertrand (*5*)). Dots represent results of the present work, and crosses those of Bertrand

EXPERIMENTS IN PRACTICAL APPLICATION

The experiments with the practical application of the chloropicrin have had to do very largely with the technique of application. In general, it has been found that the material gives a quicker and more uniform action when it is atomized. A pressure sprayer which would throw a fine mist has been considered the most satisfactory means of application.

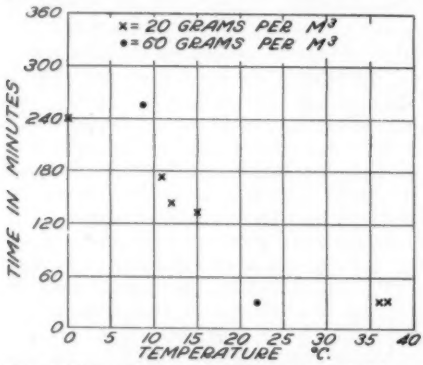

FIG. 2.—Time and concentration curves for toxicity of chloropicrin to *Calendra oryza* L. and *Calendra granaria* L.

No satisfactory method of measuring the rate of penetration of chloropicrin has been found. However, chloropicrin has been observed to have unusual powers of penetration. The confused flour beetle has been killed in the center of a 98 pound sack of flour in less than 24 hours, with a concentration of about 32 gms. per cubic meter in a wooden box. This same property, however, makes it possible for the chloropicrin to escape through the walls of the fumigation chamber more readily than other fumigants do.

After experiments in 1920, Burkhardt (*10*) concluded that chloropicrin was unsatisfactory for fumigation on a large scale, but he used only 10 gms. per cubic meter, a lower concentration than that

used by Moore and in this present work. Furthermore, in one case he merely poured the chloropicrin over sacks of grain, after which the pile was covered with a tarpaulin. It is not surprising that the small amount of chloropicrin used was soon reduced by diffusion to the point where it failed to kill.

In the course of these experiments, a room 80 by 18 feet was fumigated to kill the Indian-meal moth, *Plodia interpunctella* Huebn. The walls of the room were cracked, and the larvae had gone into the cracks to pupate. All of the walls and ceiling and floor were sprayed with chloropicrin at the rate of about 32 gms. per cubic meter for the volume of the room. The adult moths dropped to the floor as soon as they were touched by the spray, and the larvae and pupae were subjected to an atmosphere saturated with chloropicrin at the start. All the insects were killed. The room was aired by means of an electric fan placed in the only window.

In another case, the empty grain bin of an elevator which was infested with the granary weevil was treated in the same way. Here again the insects were in cracks in the wall. This method subjected them all to an atmosphere which was practically saturated with chloropicrin at the start, which resulted in their death in a very short time. At the same time the concentration was high enough in all parts of the bin to kill any insects which failed to come into contact with the liquid.

When infested grain was placed on the floor of an open box and sprayed with chloropicrin, the insects actually hit by the spray were killed while others 2 feet above the floor survived.

From these experiments it seems evident that, by virtue of its penetration property, chloropicrin is able at the same time to penetrate materials which contain insects, and to escape through the walls of the fumigation chamber so rapidly that its concentration may soon fall below the lethal point. When the material is applied as a spray, the surface of the chloropicrin is so increased that it volatilizes very quickly, thus building up a high concentration in the atmosphere before it begins to escape. Also, the spray can be directed to the infested areas, thus giving the insects a saturated atmosphere at the start.

EFFECTS OF CHLOROPICRIN ON FUMIGATED CEREALS

In the use of any material as a fumigant for cereals, not only are its toxic effects on the infesting organism and its freedom from fire hazard of importance, but worthy of equal consideration are its effects, physical and chemical, on the material fumigated. When wheat or flour which is to be used in making bread is the particular cereal fumigated, these effects may be differentiated into at least two phases. The first of these concerns the effects of the fumigant on the colloidal nature of the proteins of the flour as indicated by a physico-chemical study of such proteins. The second has to do with the effects on yeast activity, in particular on the rate of production of carbon-dioxide gas by the yeast organism. In this paper the use of chloropicrin as a fumigant will be considered from the several angles indicated.

As a fumigant for weevil-infested wheat and flour, chloropicrin has been used to some extent, but previous observations as to its effects on these materials have been of general nature and hence it seemed desirable to undertake a more critical study. Moore (*16*) observed that chloropicrin had some effect on bread prepared from chloropicrin-fumigated flours. His data tended to show that yeast activity was retarded in the fermenting dough, and that the gluten was affected to some extent, for he noted that the texture of the bread baked from treated flours was slightly inferior to that made from untreated flour.

Ryo Yamamoto (*22*) stated that when chloropicrin was sprayed into a storehouse used for rice and wheat, noxious insects were killed without bad effects on the cereals. That the fumigant had considerable penetrating ability is indicated by the fact that it killed pupae in cocoons. Piutti (*17*) stated that chloropicrin gave excellent results in killing insects with no injurious effects on wheat, flour, or bread. Bertrand and Rosenblatt (*4*, *9*) found that very small amounts of chloropicrin retarded and even inhibited enzyme action in general and fermentation in particular. It seems to the writers that this retardation of zymase activity is of importance in bread making.

EFFECT OF YEAST ACTIVITY

Bertrand and Rosenblatt (*9*) presented data which showed that 6 milligrams of chloropicrin completely inactivated zymase in a liter of the stock yeast suspension which they were using. These workers measured the activity of the enzyme in terms of sucrose which disappeared from the medium.

The manifest function of yeast in bread making is the production of carbon-dioxide gas and aeration of the dough. In this work, the carbon dioxide produced in unit time rather than the percentage of sucrose which was fermented was taken as the measure of yeast activity. A stock solution was prepared containing the following materials: 1,000 c. c. water, 30 gms. yeast, 100 gms. sucrose, and a trace of dibasic ammonium phosphate. To 50 c. c. aliquots of this solution were added varying quantities of aqueous saturated chloropicrin solution, as shown in Table I and II. This aqueous chloropicrin solution was in contact with liquid chloropicrin at 18° C., and as chloropicrin is soluble in water to the extent of 1.65 gms. per liter at this temperature, each cubic centimeter of aqueous chloropicrin solution contains 1.65 mgms. of chloropicrin. Portions of the suspensions containing the several quantities of chloropicrin were placed in fermentation tubes, and the quantity of carbon dioxide read at hourly intervals. The relative quantities of carbon dioxide are given in millimeters of tube length, the fermentation tubes not being graduated. The fermentation was carried on at 28° C.

It was thought desirable in practical fumigation to use a solution of equal quantities of carbon tetrachloride and chloropicrin. Such a solution was prepared, and to it was added distilled water. Quantities of this aqueous carbon tetrachloride-chloropicrin solution were added to 50 c. c. aliquots of the stock yeast solution. The results are given in Table II.

TABLE I.—*Effect of chloropicrin on yeast activity, as indicated by the quantity of carbon dioxide evolved*

Aqueous chloropicrin solution per 50 c. c. yeast suspension	Chloropicrin	Height of column of CO_2, in millimeters					Aqueous chloropicrin solution per 50 c. c. yeast suspension	Chloropicrin	Height of column of CO_2, in millimeters				
		1 hour	2 hours	3 hours	4 hours	5 hours			1 hour	2 hours	3 hours	4 hours	5 hours
C. c.	*Mgms.*						*C. c.*	*Mgms.*					
Control		93	225	338	425	----	1.50	2.475	30	66	93	133	----
0.1	0.165	65	160	260	367	----	2.00	3.300	18	41	58	72	----
.25	.412	62	150	232	327	----	3.00	4.950	5	10	15	19	40
.50	.825	54	140	230	327	----	5.00	8.250	0	0	0	----	0
.75	1.237	51	140	210	279	----	10.00	16.500	0	0	0	----	0
1.00	1.650	37	80	152	203	----							

TABLE II.—*Effect of aqueous carbon tetrachloride-chloropicrin solution on yeast activity, as indicated by the quantity of carbon dioxide evolved*

Aqueous carbon tetrachloride-chloropicrin solution per 50 c. c. yeast suspension	Height of column of CO_2, in millimeters					Aqueous carbon tetrachloride-chloropicrin solution per 50 c. c. yeast suspension	Height of column of CO_2, in millimeters				
	1 hour	2 hours	3 hours	4 hours	5 hours		1 hour	2 hours	3 hours	4 hours	5 hours
C. c.						*C. c.*					
Control	95	240	377	480	----	4.0	28	62	87	100	----
0.2	70	185	315	402	----	5.0	5	30	58	70	83
.5	55	155	266	385	----	7.0	0	4	10	13	25
1.0	50	145	226	296	----	9.0	0	0	0	0	0
2.0	42	124	232	355	----	12.0	0	0	0	0	0
3.0	35	100	160	218	----	15.0	0	0	0	0	0

Tables I and II show the retarding effect of chloropicrin on yeast activity in all the concentrations used. An aqueous solution of carbon tetrachloride had no effect in decreasing the rate of fermentation, hence the retardation and complete inactivation of the enzyme in the case of aqueous carbon tetrachloride-chloropicrin solution was apparently due to the chloropicrin alone. The lesser toxicity of these solutions may be ascribed to a smaller quantity of chloropicrin in the water layer when used with carbon tetrachloride as compared with a saturated solution of chloropicrin alone. In the cases in which aqueous chloropicrin solutions were used, 5 c. c. (8.25 mgms. of chloropicrin) was sufficient to completely stop yeast activity in 50 c. c. of a 3 per cent yeast suspension. When aqueous carbon tetrachloride solutions were used it required 9 c. c. to effect this result.

EFFECT OF CHLOROPICRIN ON GLUTEN

As the literature contained no information concerning the effects of chloropicrin on the proteins of flour, a series of experiments was carried out to determine some of these effects. (Results in Table III.) For purposes of comparison, glutens were prepared from an untreated flour following the methods of the A. O. A. C. (*1, p. 169*). These glutens were normal in every respect.

TABLE III.—*Effect of chloropicrin on glutens washed from treated flours*

No	Treatment	Wet gluten per 100 gms. flour	Dry gluten per 100 gms. flour	Water held by gluten from 100 gms. flour
		Grams	*Grams*	*Grams*
1	Control, untreated	36.3976	12.5760	23.8216
2	do	36.3424	12.5160	23.8264
3	Treated with chloropicrin vapor and aerated	33.5232	12.3352	21.1880
4	Treated with chloropicrin vapor and not aerated	31.5992	11.9352	19.6640
5	do	34.8200	13.6784	21.1416
6	Treated with aqueous chloropicrin solution	30.4440	11.6880	18.7560

For the study of the effects of the chloropicrin on the gluten, portions of this same flour were treated in several different ways and glutens were washed from these treated flours. The gluten represented by No. 3 in Table III was prepared from a flour treated in the following manner: 700 gms. of the control flour was placed in a 3,000 c. c. Erlenmeyer flask, and chloropicrin soaked up in filter paper was placed above the flour, care being taken that no liquid chloropicrin should come in contact with the flour. The flour was allowed to remain in the flask for 3 weeks. At the end of this time the flour was removed from the flask, spread out in a thin layer, and breezed with an electric fan for 9 hours. The flour was exposed to the air for 24 hours longer, and then portions of it were used for the experiment.

A comparative study of the gluten washed from this treated flour and that washed from the control flour indicated that the quantity of dry gluten obtained from the two flours was approximately the same—12.5 and 12.3 gms. of dry gluten from 100 gms. each of the control flour and the treated flour, respectively. There was, however, a significant difference in the quantity of moist gluten obtained from these two flours, the moist gluten prepared from the control flour having the greater weight. This meant that the chloropicrin treatment affected the water-holding capacity of the flour. Hence, comparing these same two flours again, the gluten prepared from 100 gms. of the control flour was capable of holding 23.8 gms. of water, while that prepared from the same quantity of treated flour held but 21.2 gms.

Nos. 4, 5, and 6 in Table III show the same effect of the chloropicrin in varying degrees. Nos. 4 and 5 represent glutens washed from nonaerated chloropicrin-treated flours, while No. 6 represents the gluten washed from the control flour which had been doughed up with 13 c. c. of aqueous chloropicrin solution.

The most notable effects on the gluten are, however, not evident in the numerical values which have been given. These effects are physical. The glutens prepared from the untreated flour are elastic and tenacious, while those washed from treated flours lack these properties so desirable in gluten.

It was suggested that the chloropicrin might change the hydrogen-ion concentration of the doughs from which the glutens were washed; and since it was known that changes in hydrogen-ion concentration effect a dispersion of the gluten, this factor was investigated. It was found, however, that chloropicrin effected no significant change in hydrogen-ion concentration of dough suspensions, hence the chloropicrin must owe its deleterious action on the gluten to some other property.

These effects of the chloropicrin on the properties of the gluten were shown by other methods. Twenty-gram quantities of the flours were washed first with 1,000 c. c. and then with 500 c. c. of distilled water, and the viscosity determined in the manner outlined by Gortner and Sharp (*14*). The control flour at this concentration showed a viscosity of 197° McMichael. Two treated flours gave values of 146° and 151°, respectively. This again bears out the fact that the chloropicrin affects the ability of the gluten of flour to imbibe water from a weak acid solution.

In order to measure any effects on the elasticity or tenacity of the gluten or rather of the dough, certain experiments were carried out with the Chopin (*11*) extensimeter. In the use of this apparatus, 300 gms. of flour is made into a dough with 5 per cent sodium-chloride solution. Portions of this dough are pressed into a thin layer between metal plates. This thin sheet of dough is held in place so that air can be introduced from below. Under air pressure, the dough can be blown into a thin membrane which takes the shape of a bubble. The size of the bubble so formed indicates certain properties of elasticity of the dough.

Samples of treated and untreated flour were tested in this manner. The untreated flour gave a value of 16.26. Two samples of the same flour fumigated with chloropicrin gave values of 13.8 and 13.2, respectively.

From the data, it is evident that chloropicrin affects the properties of gluten to a marked degree. However, the flours were given drastic fumigation treatment, probably much more severe than they would receive in a mill or elevator. It appears that the chloropicrin is retained rather tenaciously by the flour. This is shown by the fact that when treated flour having no odor of chloropicrin is mixed with water the chloropicrin is set free as indicated both by odor and by its effect on the eyes. Probably the best method to determine whether flour has been treated with chloropicrin is to taste it. A sharp, biting taste will indicate the presence of chloropicrin even where any analytical method known would probably give negative results.

BREAD-MAKING EXPERIMENTS

Attention will now be directed to the effects of chloropicrin on flour and yeast in mixture as used in bread making. The baking procedure is that recommended by Bailey (*2*), 450 gms. flour being used in the preparation of the dough, one-third of which is subsequently placed in the expansimeter. Table IV gives the results and Figure 3 shows sections of the bread so prepared. The bread shown in Figure 3 will be referred to as the series of July 13, 1923. The No. 1 bread was made from the untreated control flour.

A portion of the same flour from which gluten No. 3 in Table III had been prepared was used in this baking series. It had, however, been lying spread out in a thin layer for two weeks, and glutens prepared from it at the end of this additional exposure to the atmosphere compared quite favorably with those prepared from the untreated flour. Also, the bread baked from this excessively treated flour compared favorably with that baked from the untreated flour. These observations seemed to indicate that treated flours, if given sufficient areation or exposure to the atmosphere, may recover from the effects of the chloropicrin treatment. These effects, there-

fore, must be due to the presence of the chloropicrin in the flour. A section of the bread baked from this flour is shown in Figure 3, No. 2.

TABLE IV.—*Baking tests on chloropicrin-treated flours, series of July 13, 1923*

No.	Treatment	Time to the oven	Loaf volume	Expansimeter
		Minutes	*C. c.*	*C. c.*
1	Control	295	1,720	860
	Same flour Aug. 15, 1923	280	1,580	930
2	Treated with chloropicrin and aerated	330	1,750	830
	Same flour, Aug. 15, 1923	280	1,520	940
3	Treated with chloropicrin and aerated to less degree than No. 2	325	1,440	650
	Same flour, Aug. 15, 1923	260	1,590	980
4	Treated with chloropicrin and aerated to less degree than No. 3	385	600	200
	Same flour, Aug. 15, 1923	265	1,520	730
5	10 c. c. aqueous chloropicrin added to the dough	305	1,780	750
6	20 c. c. aqueous chloropicrin added to the dough	365	1,500	700
7	30 c. c. aqueous chloropicrin added to the dough	365	750	310
8	Treated with chloropicrin and insufficiently aerated	365	600	280

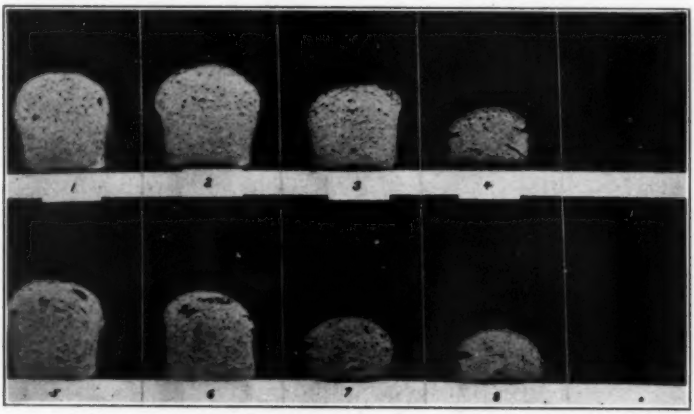

FIG. 3.—Baking tests on series of July 13, 1923: No. 1, control flour; Nos. 2, 3, 4, 8, chloropicrin-treated flours, arranged in order of degree of aeration, No. 2 having been subjected to the most aeration; Nos. 5, 6, 7 c. c. doughs treated with 10, 20, and 30 c. c. of aqueous chloropicrin solution, respectively

Considerable difficulty, however, attended the removal of the chloropicrin from the flour, as is indicated by the following experiment. A sack of the control flour (about 2 kilograms) was suspended over an excess of liquid chloropicrin, and removed after three weeks' exposure to the vapors. This sack of flour was allowed to remain out of doors for 16 hours. A portion of this flour was placed in a Mason jar, which was then stoppered, and another portion was spread out in a thin layer and left exposed to the atmosphere for 24 hours. At the end of the 24-hour period bread was prepared from portions of each of these flours. These breads are shown in Figure 3, Nos. 3 and 4, No. 3 being the one which was given the greater exposure. The bread prepared from both these flours was considerably inferior to that prepared from the control flour both as regards volume and texture. The flour which was given the greater chance to allow the chloropicrin to evaporate gave the more desirable loaf of bread.

In the preparation of the bread represented by Nos. 5, 6, and 7 in Figure 3, 10, 20, and 30 c. c. of aqueous chloropicrin solution were added, respectively. As the quantity of added chloropicrin increased, the volume of the resultant loaf of bread decreased. Thus, bread No. 7, in the preparation of which 30 c. c. of aqueous chloropicrin solution was used, attained a volume of only 750 c. c. as compared with 1,720 c. c. for the control flour. The addition of 10 c. c. of the chloropicrin solution did not markedly affect the volume of the loaf, but it did lower the expansimeter reading to 750 c. c. as compared with 860 for the control.

No. 8 shows the bread prepared from the control flour fumigated for 16 hours and aerated with an electric fan for 4 hours. The effects of the chloropicrin on the bread are even more marked than in the bread to which 30 c. c. of aqueous chloropicrin had been added. The volume of the bread prepared from this fumigated flour was 600 c. c., and the expansimeter reading 280 c. c.

The data which has just been presented indicate in a definite manner the detrimental effects which small quantities of chloropicrin have on the ability of flour to produce a good loaf of bread. Even in cases in which a fairly good loaf of bread was produced, the effect of the chloropicrin in retarding fermentation, i. e., in increasing the fermentation period, may be noted. In case of bread No. 7, in which 30 c. c. of the chloropicrin solution (50 mgms. of liquid chloropicrin) was used, activity of the yeast was almost stopped. The effects were even more marked in Nos. 4 and 8, in which the treated flours were not sufficiently aerated. In none of these treated flours was there olfactory or lachrymal indication of the presence of chloropicrin. On preparing the dough, however, the presence of the fumigant could not be doubted, the wetting of the flour setting free the chloropicrin. Tasting resulted in the same conclusion, that chloropicrin was present.

It was thought that if the flours were given sufficient time the chloropicrin would vaporize and leave them unharmed. Evidence for this belief is found in the fact that flours which at one time gave glutens of very poor quality seemed to recuperate and later give glutens of more desirable properties. Flours Nos. 1, 2, 3, and 4 of the series of July 13 were stored in cloth sacks until August 15, when bread was again made from them. Nos. 1, 2, 3, and 4 refer to portions of the same flours as were used on July 13, 1923, the first being the check flour and the last three being flours treated with chloropicrin. Table IV and Figure 4 show the results.

These data show complete recovery of the flours from the effects of chloropicrin, as far as the baking qualities were concerned. Flour No. 3, on July 13, gave a volume of 1,440 c. c. and an expansimeter reading of 650 c. c., as compared with 1,590 c. c. and 980 c. c., respectively, one month later; while for flour No. 4 the recovery is even more evident. On July 13 this flour gave a loaf volume of 600 c. c. and an expansimeter reading of 200 c. c.; on August 15, a portion of the same flour gave a loaf volume of 1,520 c. c., and an expansimeter reading of 730.

EFFECTS OF CHLOROPICRIN ON WHEAT

A series of experiments with fumigated wheats was carried on. For purposes of comparison, the wheat which was to be used in the fumiga-

tion studies was milled into flour, and bread was made from the flour. A section of the bread baked from this flour from untreated wheat is designated No. 5 in Figure 4. In Table V and Figure 4 the reference numbers indicate wheats which were treated in different ways.

TABLE V.—*Baking tests on flours milled from treated wheats, series of August 15, 1923*

No.	Treatment	Time to oven	Volume of loaf	Expansimeter
		Minutes	*C. c.*	*C. c.*
5	Control	280	1,620	860
6	Treated with chloropicrin vapor and aerated	270	1,480	900
7	Excess of chloropicrin vapor and aerated	300	750	200
8	Excess of chloropicrin vapor held 7 days	300	825	240
9	Treated with CS_2 vapors and held 16 hours	245	1,200	700

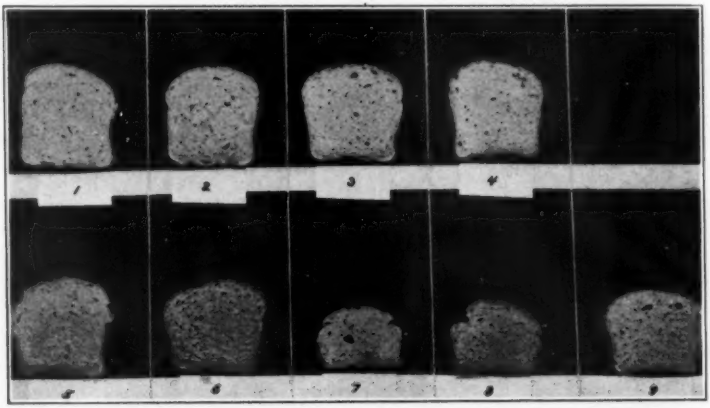

FIG. 4.—Baking tests on series of August 15, 1923. No. 1, control flour; Nos. 2, 3, 4, baked from portions of the same lots of flour as were used for the same numbers in the July 13 series; No. 5, control flour; Nos. 6, 7, 8, fumigated wheats given various degrees of aeration; No. 9, a CS_2 treated flour

Five thousand grams of the wheat was placed in a jar of 8-liter capacity and exposed to the vapors from 0.38 c. c. of chloropicrin. After a period of one week, the wheat was removed from the jar, put in a burlap bag, and hung in the mill. Sixteen hours later one-half of it was milled into flour, and on the following day bread was baked from this flour. This bread was inferior to that prepared from flour milled from untreated wheat, both as regards loaf volume and texture. The volume of the bread from the treated-wheat flour was 1,480 c. c. as compared with 1,620 c. c. for the bread made from the untreated-wheat flour.

Another 5,000-gram portion of this wheat was placed in an 8-liter jar and exposed to the vapors from an excess of liquid chloropicrin for a period of one week. It was then removed from the jar, placed in a sack, and 16 hours later one-half of it was milled into flour. On the following day, the flour was baked into bread. A similar portion of wheat was treated in the same way, except that it was milled into flour one week after it had been removed from exposure to the chloropicrin. Sections of the bread prepared from these treated-

wheat flours are designated in Figure 4 as Nos. 7 and 8, respectively. Bread No. 7 gave a loaf volume of 750 c. c., and an expansimeter reading of 200; while bread No. 8, prepared from treated wheat, which had been given a greater time to recover from the chloropicrin treatment, gave a volume of 825 c. c., and an expansimeter reading of 240 c. c. Since a tendency of the treated wheats to recover from the chloropicrin treatment was noted, portions of these wheats were retained and used in later experiments.

The data which have been given showed that even with the extensive manipulation and aeration of the wheat and its products during the milling process, the chloropicrin was not sufficiently well removed from the flour to insure good results in the bread made from them. Glutens washed from flours milled from these treated wheats showed the same inability to hold water, and the same tendency to be inelastic and noncoherent as was shown by chloropicrin-treated flours.

In connection with the detection of chloropicrin in wheat, the remarkable penetrating power of the fumigant was observed. When a few chloropicrin-treated wheat grains were placed in the mouth, no taste of chloropicrin was noted. If, however, these grains were broken up by the teeth the presence of chloropicrin was unquestionably indicated.

The presence of bread No. 9 in the August 15 series was only incidental. This bread was prepared from the control flour which had been treated with carbon disulphide and then removed from the container 16 hours before it was baked into bread. The volume of this loaf of bread was only 1,200 c. c. as compared with 1,580 c. c. for the untreated control flour. That carbon disulphide may affect the bread made from flours fumigated with it appears to be quite certain.

It has already been shown that flours can recover from the effects of the chloropicrin treatment if exposed to the atmosphere for a sufficient time. It was therefore reasonable to expect the wheats to recover in like manner. Table VI and Figure 5 give the results of experiments with treated wheats which were held in storage for a period of time before being milled. The series of experiments shown in Figure 5 will be referred to as the series of September 7, 1923. No. 1 shows a section of the bread prepared from the same control flour as has been used as a standard throughout this work, and No. 5 shows the bread made from the flour milled from the untreated wheat.

The breads designated as Nos. 2, 3, and 4 were prepared from flours milled from wheat exposed to chloropicrin vapors for 24 hours. The wheats were given 10 days to recover before they were milled into flour. Even wheat No. 4, which was treated so that liquid chloropicrin was present throughout the fumigation period, gave a loaf of bread the volume of which was 1,560 c. c. This compares quite favorably with the loaf volume of 1,620 c. c. shown by the control-wheat bread. Bread No. 3, treated with the vapors from 0.38 c. c. of chloropicrin, had a loaf volume identical with that of the control-wheat bread.

Breads Nos. 6, 7, and 8 were prepared from flour milled from portions of the same wheats as were used in the series of August 15. The quality of the bread baked on September 7 indicates that the wheat, even that treated with excess of chloropicrin, has almost entirely recovered from the effects of the chloropicrin treatment.

Bread No. 6 had a loaf volume of 1,480 c. c. on August 15 and a volume of 1,810 c. c. on September 7; bread No. 7 had a volume of 750 c. c. on August 15 and a volume of 1,700 c. c. on September 7, and bread No. 8 had increased from a volume of 825 c. c. on August 15 to 1,560 c. c. on September 7.

TABLE VI.—*Baking tests on flours milled from treated wheats, series of September 7, 1923*

No.	Treatment	Time to oven	Volume of loaf	Expansimeter
		Minutes	*C. c.*	*C. c.*
1	Control	355	1,560	760
2	Treated wheat given 10 days to recover before being milled	335	1,600	800
3	The same, 0.38 c. c. chloropicrin	330	1,620	830
4	The same, excess chloropicrin	325	1,560	850
5	Control wheat	335	1,620	760
6	Same flour as No. 6, Aug. 15	350	1,810	740
7	Same flour as No. 7, Aug. 15	345	1,700	740
8	Same flour as No. 8, Aug. 15	335	1,560	820

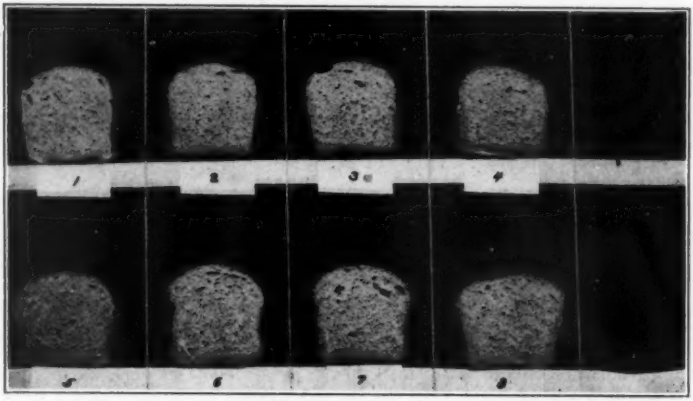

FIG. 5.—Baking tests on series of September 7, 1923: No. 1, control flour; Nos 2, 3, 4, wheats given 10 days to recover from fumigation before being milled; No. 5, flour milled from control wheat; Nos. 6, 7, 8, wheats held over from August 15 series and milled for this baking

The data obtained from these experiments again indicate the ability of the wheat to recover from the effects of the chloropicrin treatment. The recovery is more rapid in the case of wheat than it is with flour. The explanation probably lies in the greater absorptive power of the finely divided flour for chloropicrin as compared with that of the wheat.

CONCLUSIONS

Chloropicrin is highly toxic to insects.

The factors of time and temperature bear an inverse ratio to each other when concentration varies from 1 gm. to 125 gms. per cubic meter.

When concentration is constant, the time required to kill bears a linear relationship to temperature, and this relationship continues down to 0° C.

Under practical conditions, it is necessary to increase the volatility by atomizing the chloropicrin in order that a lethal concentration may be built up in the atmosphere before the loss due to leakage so reduces the amount of chloropicrin present that a lethal concentration can never be attained.

When the spray of chloropicrin is directed toward the infested areas, the insects are exposed to a saturated atmosphere while the entire volume of the room very quickly attains a lethal concentration.

When chloropicrin is present in the flour, deleterious effects are noted in the bread produced from such flour.

These effects are due to the ability of the chloropicrin to retard fermentation and to affect in a harmful manner the physical condition of the gluten-forming proteins.

When flours or wheats treated with chloropicrin are exposed to the atmosphere for sufficient time, the chloropicrin disappears from them.

Fumigated flours given proper exposure to the atmosphere show complete recovery from the chloropicrin treatment.

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OBSERVATIONS ON THE HIBERNATION OF GROUND SQUIRRELS[1]

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INTRODUCTION

While making observations on the life history of the Columbian ground squirrel (*Citellus columbianus columbianus*) difficulty was experienced in obtaining data on the hibernation. Information on this subterranean phase of the animal's life cycle was obtained, however, by patient and long-continued search and excavation in the field. But there still remained many hidden and obscure points, especially in regard to the day-by-day activity of the animal while in the state of aestivation and hibernation, which could be learned only by observations repeated continuously during the entire period of coma.

To make these observations possible it was necessary to have the animals under partial and absolute control. This was accomplishd by carefully planned and properly constructed yards for observations of a semirestricted nature and a cabin and hibernation cellar for closer and more detailed observations. For the purpose of the former, the undisturbed dens of wild squirrels were securely fenced in.[2] For the latter, a cabin (fig. 1) was constructed with which were connected small yards and beneath which was a cellar furnished with small compartments in which aestivating and hibernating squirrels could be placed and be easily observed, day or night, at the will of the observer. With this equipment, wild squirrels were obtained from the fields and the work was begun, with the following results.

As might have been expected, the sudden transfer from wild to captive life caused the squirrels to break the regularity of their life routine, and it was usually some weeks before they overcame their fear of the new surroundings and went into the comatose condition. Indeed, if food were continually furnished them they would sometimes remain awake during the entire winter.

To induce them to go into hibernation, food of fattening properties, such as they were accustomed to obtain under wild conditions, was approximated in the form of a sheaf of wheat or oats and the seeds of sunflowers. Moisture was given in the form of carrots and apples, of both of which they are very fond.

Besides those placed in the hibernation cabin, others were confined in the yards of a nearby brood cabin used for determining other phases of the life history. Here, as in the former place of confinement, they usually dug small dens in the accompanying yards and there transferred the nest. This was desirable, as it removed them from the effect of the unnatural condition of midday heat in late summer and

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[2] SHAW, W. T. A LIFE HISTORY PROBLEM AND A MEANS FOR ITS SOLUTION. Jour. Mammal. 6:157–162, illus. 1925.

also from the disturbing conditions attending feeding and the care of the yards. In the hibernation cabin in many cases they went into hibernation in the nests provided for them in the cool underground boxes. In fact, these seemed so well adapted for them that in the summer of 1915 a squirrel in one of the adjoining yards took up his abode in one of these nests which he had succeeded in reaching through a defective partition.

Those squirrels which had gone into hibernation in the earth of their respective small yards were dug out of their nests, weighed, and transferred to the nest boxes in the hibernation cabin. Here they were visited almost daily and their condition and weekly weight noted.

The records of females Nos. 7 and 8 and of males Nos. 8 and 9 are given here, showing the activities and inactivities as they occurred and giving the weights from time to time. Also the record of a Townsend squirrel is given as illustrative of some points not so well shown by the more timid Columbian.

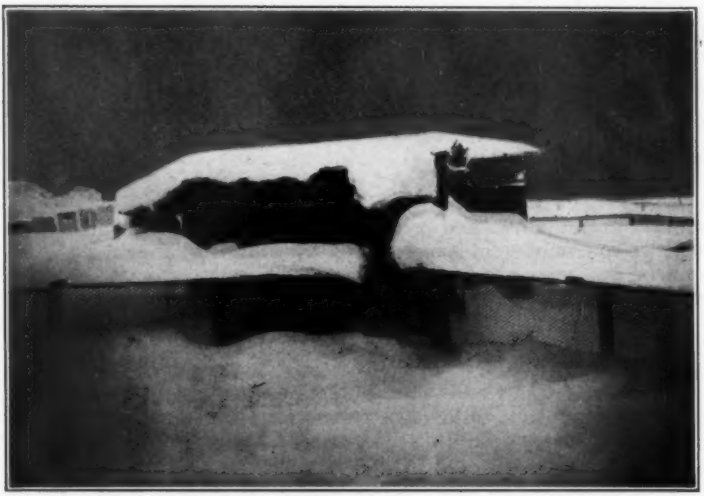


FIG. 1.—Hibernation cabin in winter. Sometimes, in the dead of winter, the cabins became all but covered with snow. Then were presented true conditions of hibernation. Body temperatures were taken, showing a fall in temperature to 40° F., at which time the squirrels were but little higher in temperature than their surroundings

HIBERNATION RECORDS

The hibernation records of female 8, showing her behavior during the winter, are given as follows: Female 8 was captured August 16, 1911, while in wild aestivation. On that date she weighed 334 grams. On being placed in yard 11, she awoke from aestivation and remained awake until September 18. Meantime, on August 21, she began the construction of a den in the earth of the outer yard. On August 23 she moved all of her nest from the box out into the newly constructed burrow in the outer yard. From September 18 to November 10 she was allowed to remain in undisturbed hibernation. On November 11 she was dug from her nest and placed in the box of yard 11.

On this date she was entirely in hibernation, but showed signs of wakening. She was observed almost daily from November 12, 1911, to March 13, 1912. Her subsequent behavior is as follows:

She was awake and warm on November 12, 13, 23; December 7; February 23, 24; March 6, 7, 8, and 12. The hibernation period ended March 12.

Her weights were as follows:

	Grams		Grams
Nov. 18	466	Jan. 20	413
Dec. 2	450	Jan. 27	409
Dec. 9	441	Feb. 4	408
Dec. 16	441	Feb. 17	399
Dec. 30	425	Feb. 25	385
Jan. 6	420	Mar. 2	387
Jan. 13	420	Mar. 9	370

This squirrel was awake on five different occasions during the winter. She lost 90 grams in weight between November 18, 1911, and March 9, 1912. She was deprived of all food from November 10 to March 13.

INTERMITTENT AWAKENING FROM HIBERNATION

As was shown by female 8, squirrels were wakening at intervals during the winter. Very careful daily observations relative to their condition revealed this fact. Males 8, 9; female 7, and *Citellus townsendi*, male 5, all showed the same characteristic of interrupted hibernation.

That they can pass in and out of the hibernation state rather easily is shown by *Citellus townsendi* No. 3, which, on November 8, was awake, November 9, in hibernation, and November 10 awake again. It was noted while making the daily observations that the squirrels would show signs of life and become somewhat warm occasionally without actually awakening.

OBSERVATIONS ON AN AWAKENING SQUIRREL

These animals are very lifeless in appearance and form when in the condition of hibernation. The eyes are very sunken and tightly closed. The mouth and lips are formless as in death. No sign of life remains, save a possible slow movement over the flanks, during which period the hairs of the region are slowly caused to bristle in erection. One in profound hibernation was removed to a warm room, and as she began slowly to show signs of life the following observations were made of her:

At 3.20—Rhythmical thoracic respiration movements were noticeable. Mouth was opened slightly.

At 3.32—Squirrel began to rub paws over her nose. Eyelids began to twitch. Body began to straighten out, and the front legs began to work in a writhing motion, from side to side, over the chest, the paws being held together about the middle of the belly.

At 3.40.—This action was speeded up. The motion was fairly regular, suggesting the beating of the heart (the alternate contraction of auricle and ventricle).

At 3.50.—Change took place. Up to this time very little or no movement of the hind legs and the hinder part of the body, but now the hind legs began to move. At this time, owing to the lateness of the day and failing photographic light, the squirrel was warmed by artificial heat.

At. 3.55.—Regular respiratory movements commenced, accompanied by muscular movements of legs and back. Animal straightened out. Hitherto she had been lying on her side, trembling like a new-born lamb, and apparently unconscious and helpless. Now her front limbs were shaking like one with the ague, while the hind limbs were perfectly still.

At 4.05.—She was gasping for breath.

At 4.10.—Violent shaking of the front paws, with a straightening out of the body. There was still little motion of the hind limbs.

At 4.22.—She was able to maintain a position on her belly, attempting to crawl. Eyes still shut.

At 4.25.—Rapid heart beat noticeable. For the first time the muscles began to be resistant. There was quite a tenacious grip in the toes. The tail was controlled by muscular movements. She responded nervously when touched.

At 4.33.—Squirrel was very warm and comfortable, and well able to crawl about. It was now difficult to keep her in front of the camera.

One very noticeable feature was the very great acceleration in the awakening process, beginning at 4.25 and terminating with the almost completely wide awake condition at 4.50.

Three other squirrels were studied, and in each case similar observations were made. In all of these it was noticed that the posterior part of the body remained inactive and lifeless much longer than the anterior. These observations were made independently, but later it was noted that a French experimenter, Marès,[3] showed by injecting ground squirrels that the circulation in the posterior part of the body was largely suspended during hibernation and that this part of the body was the last to come from that condition.

That they regain fleetness and acutenss of their senses in a short time is shown by the following incident: On November 18 a squirrel was dug from hibernation two days before it was taken back to its den to be photographed. Suddenly it made a dash for liberty and darted into a very obscure hole beside a fence post, about 3 rods from its den, seeming to be familiar with the surrounding territory and in keen possession of its senses.

Interesting observations were made during the winter in regard to daily weight losses and the duration of hibernation. The records are given in Table I. This table shows a rather constant loss of about 1 gram a day for both the Columbian and Townsend squirrels.

TABLE I.—*Weight losses, in grams, of five squirrels during winter hibernation*

Species	Weight when going into hibernation	Weight when coming out of hibernation	Total weight lost	Duration of hibernation in days	Amount lost per day in grams
C. c. columbianus male No. 8	592	530	62	63	0.98
C. c. columbianus male No. 9	445	394	51	28	1.8
C. c. columbianus female No. 7	414	273	141	77	1.8
C. c. columbianus female No. 8	500	370	130	119	1.09
C. townsendi male No. 5	280	221	59	56	1.05

[3] MARÈS, F. EXPÉRIENCES SUR L'HIBERNATION DES MAMMIFÈRES. (Mémoire) Compt. Rend. Soc. Biol. [Paris] (sér. 9, t. 4) 44: 313-320. 1892.

After awakening from hibernation the squirrels began to take on weight, as is shown by the record of female 8. On March 9, three days before the hibernation ended, she weighed 370 grams, on May 16, she weighed 411 grams, having gained 40 grams in 64 days or 0.62 gram per day, a little more than half the daily loss in the winter. There was a greater gain shown by female 4. On coming from hibernation, probably March 14, she weighed 301 grams on March 19; on May 16 she weighed 422 grams, having gained 121 grams in 58 days, or a little more than 2 grams per day. Of course, the weight on May 16 might have been increased by bulky food. Immature male 6 increased 55 grams in weight between March 30 and May 24; immature female 10 increased 86 grams between April 4 and May 12; immature male 11 increased 28 grams between March 30 and May 24.

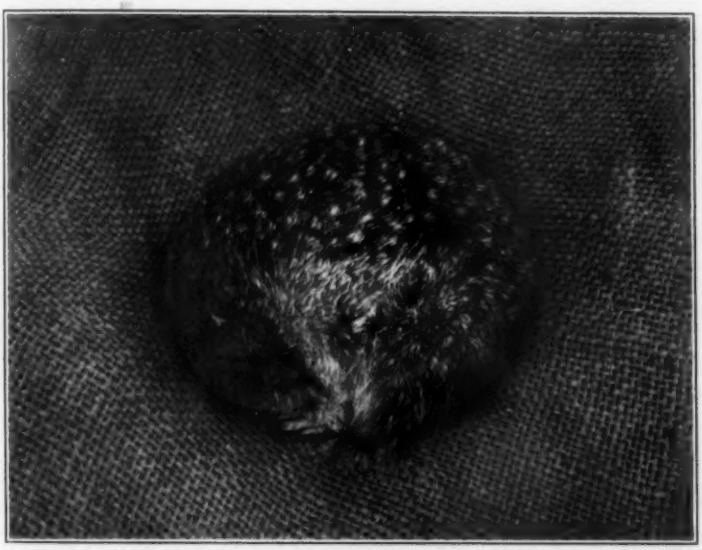


FIG. 2.—A Townsend ground squirrel in hibernation. This squirrel, brought from low elevation of 1,471 feet, went into aestivation at the remarkably early date of June 14. After an almost continuous sleep of eight months, he appeared as in Figure 3

DURATION OF HIBERNATION IN THE HIBERNATION CABIN

A very remarkable hibernation record was that of the *Citellus townsendi* male 5. For a few days prior to June 14, 1912, he had not been eating regularly and had been plugging up the cracks in the nest box and the hole in the chute leading to the yard. On June 14 he was found in aestivation (fig. 2). He passed through the usual hibernation. On January 31, 1913, he showed signs of having been awake, as he had torn down part of the plug in the exit shaft, which had been untouched since being put in by him in June. He was still in hibernation February 13, having been in a state of aestivation and hibernation for a period of 244 days or, roughly speaking, 8 months. It is possible he would have ended his hibernation in January had he been at liberty, but finding the exit

of his box closed he acted philosophically and postponed starvation by returning again to hibernation. On February 14 it was found that he had been gnawing in an effort to get out, so he was given his liberty. On this date he was fully awake and came out into the snowy yard and ate some carrot (fig. 3).

On coming from hibernation permanently they soon repair their nest. This is done by piling up the material in a neat, fresh, warm-looking structure suitable for an active life.

DURATION OF HIBERNATION IN THE LARGE YARDS

In the winter the large dens of the yards were used for hibernation studies. It was found that the duration of hibernation for two males was 169 and 131 days; for 4 females, 190, 178, 178, and 179

FIG. 3.—Yard observations. In the cellar, it was possible to observe squirrels daily the winter through, thus enabling comparison of these conditions with field conditions. This squirrel (a Townsend) slept almost continuously for eight months. He awoke at about the normal time, but, not finding food, went back to sleep

days; for 2 young, 209 and 233 days. Of course, conditions were not normal, as in the fields, and the squirrels were slow in going into hibernation. The young, being tamer, went first. As in the fields, the duration of hibernation is greater among the females than among the males, and in one case the female returned from hibernation 15 days later than the male. One male lost 123 grams in 169 days or 0.7 gram per day.

TEMPERATURE OF BOXES

Nine daily records taken between August 14 and September 9, 1912, showed that the summer temperature of the boxes was much more constant and favorable to aestivation, varying only 7 degrees, against a 35-degree range of air temperature and averaging 15 degrees lower than the air. A hibernating squirrel can resist a

temperature 4° to 6° F. below freezing about its nest. On February 3, 1914, the box temperature where the squirrels were kept was 30.5° F., resulting in no harm to the hibernating squirrels. From this one can understand why it is that young squirrels risk the danger from frost when hibernating only 6 or 7 inches below the surface of the ground.[4] It was noted also in the hibernarium that the squirrels would come out of the hibernation condition if the boxes containing them went much below the freezing point. Of course, the nest material would assist in keeping the temperature up (fig. 1).

BODY TEMPERATURE OF HIBERNATING SQUIRRELS

The body temperature of squirrels in hibernation was taken. At first this was reached approximately by placing the thermometer against the ventral side of the squirrel. Later, rectal temperatures were taken. The results are given in Table II. From this table it is seen that the average body temperature of three hibernating squirrels tested by the rectal method is 42.3° F., or about 56° below normal. It is noteworthy that in the case of the squirrel tested February 12 her temperature rose from 38° F. to normal 98° in four hours.

TABLE II.—*Body temperatures of hibernating squirrels, in degrees Fahrenheit*

Date	Kind of test	Temperature of nest box	Temperature of squirrel	Duration of test
				Min.
Jan. 20, 1912	Contact	38.5	41.0	
Mar. 1, 1912	do	34.0	38.1	
Feb. 3, 1914	Rectal	30.5	49.0	18
Feb. 12, 1914	do	36.0	38.0	6
Feb. 16, 1914	do		40.0	15

RELATION OF FEEDING TO AESTIVATION AND HIBERNATION

It was noticeable that squirrels preparing for aestivation or coming from hibernation used very little food, and this was taken at intervals of some days. For example, in August a Columbian ground squirrel was observed to have an interval of seven days between times of eating. On August 25 it partook of a very little food. This seemed to be its last meal before going into hibernation. A Townsend squirrel did not eat much for several days before going into aestivation.

That they need not eat during hibernation is shown by a Columbian under observation. It was given no food from November 1, 1911, to March 13, 1912. When they were given food while awake it seemed to interfere with the regularity of their hibernation and even to keep them out of this condition altogether. In the case of Columbian female 7, taken from the hibernation condition on November 11, 1911, she afterwards awoke and was fed November 14. She remained awake for some days, but went into hibernation November 20. She ate again December 14, and was in hibernation again on December 16. She ate once more January 1, 1912, and went into hibernation January 2. Again on January 17 she ate and went back into hibernation the following day. On January 29 she awoke and ate and did not go back into hibernation.

[4] SHAW, W. T. THE HIBERNATION OF THE COLUMBIAN GROUND SQUIRREL. Canad. Field-Nat. 39 56-61, 79-82, illus. 1925.

Another case of feeding was that of male 9, given food on December 31, 1911. He was awake and ate on January 11 and 14, after which he remained awake. *Citellus townsendi* male 5 ate sparingly the first day out of hibernation.

For squirrels to consume food at all in the winter is not normal. Occasionally in the yards a squirrel would not go into hibernation, but remained warm and active, consuming very little food. On December 11, 1913, a squirrel was tested for the amount of food consumed in a day and it was found that it ate only 8 grams of carrot and sunflower seed. It showed a preference for the seeds. On December 21 it ate 4 grams of carrot and 1 gram of sunflower seed, making 5 grams in all.

FIG. 4.—In the yards, some of the squirrels, captured late in the season and being very wild, would not enter into hibernation, and seemed to take very little food during the entire winter. In order to make observations regarding the necessity of food in winter, though awake, this squirrel was placed in hibernarium box which had been completely cleaned so that no food should remain. A warm nest of excelsior and cotton batting was provided. The squirrel was observed from day to day. During all the time of his fasting he seemed to be in good condition and wide awake. At the end of the sixteenth day without food he went into hibernation, as shown in the figure

That they can get along without food at this period is shown by the following observation: One of these squirrels, refusing to go into hibernation, was deprived of food for 16 days, during which time it remained awake, warm and lively. On the seventeenth day it went into hibernation, remaining in this condition for six days (fig. 4). At the beginning of the test it weighed 495 grams. At the end of the test it weighed only 358 grams. On awakening, it ate and continued its existence as if nothing had happened (fig. 5). That aestivation and hibernation are offsets to starvation is evident.

APPEARANCE OF A HIBERNATING SQUIRREL

Throughout all the work of hibernation it was observed that the squirrels, especially during the last of the season, became very lifeless. They took the vertical position of the squirrels shown in Figure 4, a position maintained only in a well-packed nest, and dropped to the temperature already described. They appeared less rigid during

the first few days of aestivation, as is shown by a Townsend squirrel in Figure 6. Their eyes were sunken very deeply into the orbits, and their lips became very shapeless and deathlike. Occasionally,

FIG. 5.—Same squirrel as shown in Figure 4. After going into hibernation on the sixteenth day of fasting, he slept for about six days, when he awoke and was given food. Later he was branded and turned in one of the larger outer yards where he ran all summer, regaining his normal weight

FIG. 6.—Observations in the yards have given interesting testimony on the manner in which aestivation comes over the squirrels. During the few days previous to going into the comatose condition, they gradually cease eating. Then, as in the case of the Townsend squirrel shown here, they go into torpor. They do not always curl up tightly at first, and they may waken after a day or so. At this time they get back their normal temperature, which they may retain for a short time before going back to sleep. Later in the winter, however, they appear extremely torpid, and at times it is difficult to believe they are really alive, as shown in Figure 2

after long watching, one would see a movement pass across the flank, resembling in slowness that of the peristalsis movement shown by a recently chloroformed mammal in the laboratory. From this condition they might transform in a few hours to the state of very live and warm squirrels.

THE VALUE OF CALCIUM PHOSPHATE AS A SUPPLEMENT TO THE RATION OF DAIRY COWS[1]

By J. B. LINDSEY, *Vice Director and Chemist*, and J. G. ARCHIBALD,[2] *Assistant Professor of Research Chemistry, Massachusetts Agricultural Experiment Station*

INTRODUCTION

The mineral requirements of the animal system constitute a phase of the science of nutrition which has only within recent years attained any degree of prominence. From the time when Voit and his coworkers first established on a firm basis the nutritive function of those major constituents of foodstuffs—protein, carbohydrates, and fats—up until as late as the close of the first decade of the present century, energy values and nutritive ratios were the only standards by which food values were measured. Because of the prominent place which these factors occupy in any rational system of nutrition, it was only natural, and in one sense right, that emphasis should be placed on them to the exclusion of minor factors whose significance and importance either were not realized at all, or, if realized, were but imperfectly understood.

One of these minor factors was an adequate supply, both as to quantity and quality, of the mineral elements necessary for the proper functioning of the animal system. It is true, of course, that the necessity of the presence of these inorganic constituents in the diet was long ago recognized and their functions in the body understood, but the general conception was that an otherwise normal diet supplied a sufficiency of mineral matter. It was not realized that there may be times when the supply of mineral matter in the ration becomes a limiting factor in the functional well-being of the animal. Armsby (*1, p. 332, 333*) [3] sums up the situation in the following terse sentences: "Most feeding stuffs, however, and particularly the mixed rations of farm animals, contain what appears at first sight to be much larger amounts of ash ingredients than the body requires." As a consequence the idea became somewhat prevalent "that rations adequate in other respects may be assumed to contain a sufficiency of ash ingredients. This is doubtless true of animals living in a state of nature, but it is a questionable assumption under the artificial conditions to which many farm animals are subjected."

That animals may at times suffer from a shortage of mineral matter in their rations is now well established. The present status of our knowledge on this point, and on the whole problem of mineral metabolism in farm animals, is due largely to several important researches made in recent years, and can best be outlined by a brief review and summary of these researches. In all the researches to be mentioned, the emphasis has been put largely on the metabolism of calcium and phosphorus, and especially on the former. There are

[1] Received for publication Jan. 26, 1925; issued December, 1925. Published with the permission of the Director of the Massachusetts Agricultural Experiment Station.

[2] This project was planned by the undersigned with the cooperation of Mr. Archibald. The latter supervised the execution of the experiment, holding frequent conferences with me as it progressed. Owing to my many duties, it became necessary for him to prepare this paper for publication.—*J. B. Lindsey.*

[3] Reference is made by number (italic) to "Literature cited," p. 790.

two reasons for this: (1) the important part played by these elements in the animal system, and (2) rations are more likely to be deficient in them than in any other mineral element.

Forbes (*4, 5, 6, 8, 9,*) and his coworkers at the Ohio Agricultural Experiment Station conducted a very elaborate and comprehensive series of metabolism experiments with cows in different stages of lactation. They found that high-producing cows in the earlier stages of lactation, even when fed on rations rich in calcium and phosphorus, invariably showed negative calcium balances and generally had negative phosphorus balances. This negative balance persisted until the later stages of lactation. When the milk flow had dropped to about 10 pounds daily the animals began to show positive balances of calcium and phosphorus, and from that time until calving the intake of these elements was greater than their rate of excretion. In other words, the losses of early and middle lactation were made good by storage during the later stages of lactation and in the dry period. Forbes considers this tendency on the part of milking cows to excrete their body reserves of calcium and phosphorus to be due to the intensification by breeding of the function of milk secretion to a point which is beyond the animal's ability to assimilate these elements from her feed.

As a result of his studies, Forbes recommends that cows be fed a ration naturally rich in calcium and phosphorus, i. e., one containing legume hay as a roughage, which is nothing more than a matter of good dairy practice. In addition he recommends a dry period of six to eight weeks, during which time the cow is to be fed liberally on a mineral-rich ration in order that she may make good the depleted mineral reserves of her body. He has also studied the possibility of supplying calcium in the form of mineral supplements, and his conclusion is that "it is not clear that supplemental calcium is utilized," although he suggests "for practical experimental purposes, the feeding of calcium phosphate in the form of steamed bone."

Forbes (*3, 7*) also conducted extensive experiments with swine on the value of mineral supplements in the ration. He found that pigs supplied with either precipitated calcium carbonate or special steamed bone had relatively dense and strong bones; that in pigs fed rock phosphate as a supplement the bones had about the same characteristics as those fed on a ration containing no mineral supplement, viz., relatively low breaking strength and density; that the hardest bones contained the highest proportions of calcium and total ash, while the softest bones contained the highest proportions of phosphorus and magnesium and relatively little calcium. He considered that the amounts of calcium, magnesium, and phosphorus in the bone are susceptible of much greater modification through the composition of the food than are their relative proportions. Special steamed bone proved to be the most satisfactory mineral supplement for swine of any he investigated.

Another prominent group of investigators in this field has been Hart and his colleagues at the Wisconsin Agricultural Experiment Station. Their earlier work was principally with swine. They demonstrated (*13*) that hogs are very sensitive to low ash content in their rations, that they can utilize inorganic phosphate to advantage, and that organic phosphorus (e. g. phytin) is no better than tricalcic phosphate for supplying the animal's needs for phosphorus.

Following up the investigation, they concluded (*14*) that calcium is the mineral element most likely to be deficient in ordinary rations and that swine should receive some form of supplemental calcium in addition to their grain ration.

Their work with cows on mineral metabolism has been the natural outgrowth of a most interesting series of experiments in which it was strikingly demonstrated that rations from different sources have a decidedly different biological value, regardless of how similar they may be with respect to digestible protein and net energy. In the case of rations restricted to the oat-plant, reproduction in cows was a failure, the calves being born either prematurely dead, or weak (*16*). When calcium salts, either carbonate, phosphate, or acetate, were added to the ration, a marked improvement in the reproductive function was noted.

Their more recent work has consisted of a series of mineral metabolism experiments with goats and cows, attention being centered on the assimilation of calcium (*17*). Their conclusion, as a result of four separate investigations, is that calcium assimilation is facilitated by the presence in the ration of an unidentified accessory substance which is present in green roughage and in dry roughage which has been carefully cured without exposure to direct sunlight, but not in dry roughage as ordinarily cured. They consider as a result of their work with goats (*17*) and dogs (*23*) that cod-liver oil contains the same substance. Their evidence on this latter point is strengthened by some work of McCollum et al. (*21*) who have demonstrated the existence of a fourth vitamin in cod-liver oil and other fats distinct from vitamin A and having to do with the metabolism of calcium.

Hart and his coworkers (*17*) also conclude that the ordinary grasses, even in their green state, do not furnish sufficient calcium to meet the needs of milking cows. The calcium must be supplied either in legume hay or as a mineral supplement, with the additional safeguard of some medium which carries the unidentified accessory substance essential to optimum utilization of the calcium.

Meigs, (*22*) of the United States Department of Agriculture, has also conducted a series of investigations on the subject of mineral matter for cows. His principal conclusion is essentially the same as that of Forbes, viz, that cows should be given a two-months' dry period, during which time they should be liberally fed on a ration rich in calcium and phosphorus. He noted a phenomenal increase in milk yield when cows were fed sodium phosphate with their grain ration on alternate days, and concluded that one of the principal reasons why cows often fail to maintain a high production record in successive years is because their rations are deficient in mineral matter.

The work of Welch (*24*), of Hart and Steenbock (*15*), and of Kalkus (*19*) on the relation of the iodine content of the ration to hairlessness and goiter in the newly born young of many classes of farm animals is another phase of the problem. The results of these three separate investigations have given quite conclusive proof that the prevalence of goiter and hairlessness in young animals in certain regions is due to improper functioning of the thyroid gland, caused by lack of iodine in the rations of the pregnant mothers, which can in turn be traced to lack of iodine in the soil and drinking water of

the region. The trouble can be largely corrected, if not altogether removed, by mixing small doses of potassium iodide with the feed of pregnant sows, ewes, does, cows, etc.

The foregoing researches constitute the chief contributions of recent years to the subject of the mineral requirements of farm animals. Although they have done much to advance the knowledge of the subject, there yet remain many phases of it to be investigated. The investigations with cows have for the most part been metabolism experiments, which of necessity can be conducted for only relatively short periods of time. Long-time experiments on the effect of feeding mineral supplements to milking cows are not recorded in the literature. Forbes, in his most recent paper on the subject (*8*), remarks that "The advantage to be derived from the feeding of mineral supplements seems to us doubtful, but . . . that the possibilities of benefit from so doing should be thoroughly investigated . . ." In a recent report of the subcommittee on animal nutrition of the National Research Council (*10*) occurs the following paragraph:

> An important problem in this field is to determine, by carefully controlled, long-time feeding experiments with dairy cattle, under conditions of practice, the effect of differences of intake of mineral nutrients, especially as contained in leguminous as compared with gramineous roughage, and in mineral supplements, on growth and productive efficiency.

Such an investigation has been in progress at this station for the past two and one-half years and is still being conducted. This paper constitutes a report of progress on the project up to May 1, 1924.

SCOPE, METHODS, AND RESULTS OF THE INVESTIGATION

OUTLINE OF THE PROJECT

In brief, the essentials of the investigation have been as follows:

(1) Feeding to the station dairy herd of a ration as deficient in calcium and phosphorus as it has been possible to formulate from the roughages and concentrates available, but at the same time adequate in other respects.

(2) Division of the herd into two halves as nearly identical as the individuality of the animals would permit, and the addition to the grain ration of one of these groups (always the same group) of supplemental calcium phosphate in the form of a special steamed bone meal, the amount varying from 3 to 8 ounces daily, depending on the weight and milk yield of the cow.

(3) Observation of the effects of the mineral supplement upon:

- (*a*) The general condition of the animals as apparent to the eye and as revealed by handling.
- (*b*) Body weight.
- (*c*) Milk production.
- (*d*) Composition of the milk, especially its content of calcium and phosphorus.
- (*e*) Reproduction, under which general heading have been observed:
  - (1) Recurrence of oestrum.
  - (2) Difficulties in getting cows to breed.
  - (3) Such abnormalities as abortions, retained placentas, etc.
  - (4) Condition of the calves at birth and subsequently.

METHODS OF EXPERIMENTATION

General Procedure

The station herd is made up of high-grade Holsteins and Jerseys, and usually numbers, including young stock, about 18 to 20 head. At present there are 20 animals included in the experiment, 15 milk cows, 3 yearling heifers, and 2 eight-months-old heifers.[4] There have of necessity been some changes in the herd since the experiment was started, but of the 19 individuals originally included, 13 are still in the herd. Since the commencement of the experiment in December, 1921, the milking herd has had an average annual production per cow of 8,200 pounds of milk, testing 4.52 per cent of fat. The herd is maintained for the most part by raising the heifer calves; only occasionally are any cows purchased.

All the herd is housed in a well-lighted, well-ventilated stable, and except in inclement weather receives daily exercise in adjacent yards. Up until last year all cows dry during the pasture season have been sent to pasture regularly for a period of four to six weeks. For reasons outlined later, this practice is no longer followed, only the young stock being pastured. All cows are given a two-months' dry period, during which time they are liberally fed. The aim is to have the cows freshen in the autumn whenever possible, but this is a matter which is difficult to regulate in practice. The cows are maintained on dry feed the year around, except for a period commencing about June 25 and ending about October 10, during which time they are fed such green soiling crops as oats, millet, sorghum, and fodder corn as a substitute for a portion of their hay. No ensilage or roots are fed. Running water is before the cows at all times, and salt is furnished to the extent of 0.75 per cent of the grain ration. The cows are fed and milked twice daily (at 7 a. m. and 5 p. m.); each cow's daily ration is carefully weighed out—the hay into 4-bushel baskets, the grain into suitable trays. The milk is weighed and accurate records kept of the production of each cow and the cost of the feed. Composite samples of every lot of hay and grain purchased are taken regularly, and the milk is sampled once each month for five consecutive days. The composite samples are analyzed as outlined later on in this paper.

All animals are weighed on the 1st and 2d of each month, and their rations adjusted according to their body weights and the amounts of milk they are giving. The standards for digestible nutrients employed in formulating and adjusting the rations are:

For mature cows, Haecker's standard for the aged cow (*12, p. 56–57*).
For heifers that have had one calf, Haecker's standard for heifers (*11, p. 107*).
For heifers previous to their first calving, Armsby's standard for maintenance and growth in the dairy breeds (*1, p. 713*).

The standard adopted for calcium and phosphorus requirements of all the animals has been that proposed by Kellner (*20, p. 618*), founded in part on the observations of Henneberg (*18, p. 53*) and in part on Kellner's own work. This standard calls for a daily minimum of 100 grams of calcium oxide (CaO) and 50 grams of phosphoric acid (P_2O_5) for each 1,000 kg. of live weight, for maintenance only. In addition, for each 20 kg. of milk produced, there must be supplied

[4] All heifer calves are included in the experiment as soon as old enough to subsist on hay and dry grain. The age varies with the calf from 5 to 7 months.

50 grams each of calcium oxide and phosphoric acid. Expressed as the elements (Ca and P)[5] and on a pound basis the standard is as follows: A cow weighing 1,000 pounds requires for maintenance (daily), 0.07 pound calcium, 0.021 pound phosphorus; for each pound of milk produced, 0.002 pound calcium, 0.001 pound phosphorus.

The analytical data which have been employed in adjusting the rations to the standards have been very carefully compiled from a large number of analyses and digestion studies of the several materials fed. In the case of the mineral constituents of the various feeds, it was found at the start of the experiment that comparatively few analyses had been made, so a separate project[6] was organized and the mineral constituents of a large number of samples of the roughages and concentrates fed were determined.

Rations Fed

As already stated, the ration fed has designedly been as poor in mineral matter as a wise choice of feeds, other factors being considered, would permit. It has not been possible or even desirable to feed the same identical ration at all times, but the content of digestible nutrients has been kept as constant as possible throughout. The following is an accurate account of the various modifications in the rations:

Hay.—The hay fed was all grown locally, most of it on the station farm, and it has all been of the same general character, consisting for the most part of timothy with some redtop, bluegrass, and orchard grass. In all purchases of hay, a minimum of clover has been insisted on and none of the hay fed has contained more than a very small percentage of it. Some of the lots fed have not had even a stray stalk of clover. The average amount of calcium in these hays has been 0.47 per cent and of phosphorus 0.19 per cent. In order to impoverish the ration still further with respect to calcium and phosphorus, for the past year and a half a portion of the hay has been withheld and such mineral-poor materials as chopped oat straw starch, and dried apple pomace have been successively substituted for it. The calcium and phosphorus content of these has been: Oat straw, 0.38 per cent Ca, 0.14 per cent P; starch, none; dried apple pomace, 0.11 per cent Ca, 0.11 per cent P.

Green soiling crops have been fed during the summer in place of a portion of the hay. During the first summer the experiment was in progress (1922) no departure was made from the long-established custom of feeding a maximum of 50 pounds of green feed per cow daily and of including in the succession of soiling crops such legumes as field peas and soy beans. Realizing that here might be a possible source of more calcium than would be desirable to feed under the conditions of the experiment, the amount fed was cut down last year (1923) to a maximum of 25 pounds daily, peas were eliminated from the rotation, and only a minimum of soy beans was fed. For a similar reason and as previously noted, the dry cows were not sent to pasture last summer (1923).

[5] The writers in all their work have reduced their Ca and P figures to the elemental basis, not CaO and P_2O_5.

[6] Results of this project are as yet unpublished.

GRAIN.—For the first 10 months of the experiment the grain ration consisted of—

	Pounds
Ground oats	39
Gluten meal	20
Corn meal	20
Wheat bran	20
Salt	1
Total	100

This combination contained about 15 per cent digestible protein, 55 per cent digestible carbohydrates and fat, 0.035 per cent calcium, and 0.40 per cent phosphorus.

In October, 1922, as already noted, chopped oat straw and starch were substituted for a portion of the hay. As a consequence of this change it became necessary, in order to balance the ration, to increase the protein content of the grain mixture. This was done by increasing the gluten meal to 30 pounds per hundred, and reducing the corn meal to 10 pounds. The resulting grain mixture contained about 18 per cent digestible protein, 52 per cent digestible carbohydrates and fat, 0.035 per cent calcium, and 0.42 per cent phosphorus.

The ration was again modified in September, 1923, dried apple pomace being substituted for the starch, the oat straw having been discontinued when the feeding of green fodder commenced in June. It occurred to the writers at this time (September, 1923) that possibly such a high percentage of gluten meal in the ration was not desirable, so the amount was reduced to 20 pounds, and in order to keep up the protein content 20 pounds of red-dog flour were substituted for the corn meal, so that the ration now being fed consists of:

	Pounds.
Ground oats	39¼
Gluten meal	20
Red-dog flour	20
Wheat bran	20
Salt	¾
Total	100

This combination contains about 17 per cent digestible protein, 53 per cent digestible carbohydrates and fat, 0. 06 per cent calcium, and 0.44 per cent phosphorus.

The foregoing variations in grain and roughage have not materially changed the general character of the ration, which, as already noted, has been formulated so as to be low in its content of calcium and phosphorus. Judged according to Kellner's (*20, p. 618*) standard for these elements, the rations have averaged throughout the experiment about 33 per cent deficient in calcium but have supplied a 62 per cent excess of phosphorus, thus showing that ordinary rations are much more likely to be lacking in calcium than in phosphorus, due to the relatively high percentages of phosphorus in all grains.

All the cows in the herd have received this ration, and, in addition, one-half of the herd, known as the "mineral" group, have received supplemental calcium and phorphorus in the form of a steamed bone meal, especially prepared for animal feeding. The bone meal has been added to each cow's daily grain ration at the time of weighing, being thoroughly mixed with the grain before feeding. The amount

for each cow, from 3 to 8 ounces daily, has varied with her weight and the amount of milk she gives, the aim being to supply approximately a 60 per cent excess of calcium over and above the theroetical requirements of Kellner's standard (*20, p. 618*). This excess is provided simply as a safeguard. Little is known of how completely such substances are assimilated, but it is safe to assume that they are by no means completely utilized, and just as there must be provided an excess of total organic nutrients in order to have a sufficiency of digestible organic nutrients, so there must be an excess of total mineral matter. The 60 per cent excess is an arbitrary figure.

Supplying this amount of calcium in the form of tricalic phosphate has also greatly increased the amount of phosphorus, so that the surplus of that element furnished to the "mineral" group has averaged around 160 per cent. This does not mean, however, that the ratio of acid to base in the ash of the total ration has been materially increased. The rations fed as just outlined have had a considerable excess of bases in their ash, and as the bone meal itself has an excess of base over acid, the addition of the mineral supplement, although it has relatively enhanced very much the excess of phosphorus over and above requirements, has intensified rather than minimized the basic nature of the ash.

ANALYTICAL PROCEDURE

Fodder and ash analyses of all materials fed have been made as the experiment progressed. Total solids and fat have been made monthly on the milk from every cow milking at the time, and in addition the total ash of all milks has been determined in 12 of the 30 months during which the experiment has been in progress, and over a period extending from June, 1922, to March, 1924. The calcium and phosphorus in the ash of the milks has been determined at nine different times and over a period extending from October, 1922, to March, 1924. A determination of the calcium and phosphorus in the drinking water supplied to the cows has also been made,

The methods of analysis have in all cases been those of the Association of Official Agricultural Chemists as described in the manual issued by that association (*2*).[7]

The foregoing is an accurate outline of the method of procedure in this "long-time feeding experiment with dairy cattle under conditions of practice." What are the results? After two and one-half years of continuous feeding to half the herd of a ration deficient in calcium and none too liberal in phosphorus, and to the other half the same ration plus sufficient supplemental mineral matter to provide a considerable surplus of both of these elements, what can be said either for or against the practice of supplying mineral matter in this form? The answer, so far as we have gone, is given in the next section and in the summary which follows it.

PRESENTATION AND DISCUSSION OF RESULTS

General Condition of the Animals

An endeavor has been made to follow the condition of the animals closely, and observations have been recorded from month to month.

[7] Acknowledgment is made here of the services of P. H. Smith and F. J. Kokoski of the feed laboratory and of H. D. Haskins and L. S. Walker of the fertilizer laboratory, who did practically all of the analytical work in connection with the project.

At no time has it been possible to note any marked difference in the aged cows. There have been times when, as a group, they have not been in the best of condition, but this has been true of the whole aged herd and not of any particular individual or group. Of the 14 aged cows with which the experiment was commenced, 9 are still in the herd, 4 of them being in the "mineral" group and 5 in the "nonmineral" group. (See Table I.)

TABLE I.—*History and present condition of the aged cows*

Cow No.	Age in years	Breed	Group	Number of calves		Present condition (May, 1924)
				Previous to commencement of experiment	Since commencement of experiment	
6	7	Grade Holstein	Nonmineral	1	2	Good.
8	12	do	do	[a] 1	2	Fair.
9	9	do	Mineral	[b] 1	[c] 2	Good.
10	10	do	Nonmineral	5	2	Do.
11	10	do	Mineral	4	[d] 2	Do.
12	7	do	do	1	3	Do.
16	11	Grade Jersey	Nonmineral	6	2	Do.
17	11	Purebred Jersey	Mineral	7	2	Fair.
19	7	Grade Jersey	Nonmineral	2	2	Good.

[a] This cow was purchased when 7 years old. Her calf record previous to purchase in 1920 is unknown.
[b] Purchased in 1921. Calf record previous to purchase unknown.
[c] One of these was aborted at 7 months.
[d] One of these was aborted at 5 months.

In so far as the eye can detect, none of these cows are undernourished or deprived of anything. As seen by their photographs (pls. 1 and 2), they are above the average in appearance, and even a keen observer would see nothing radically wrong with any of them.

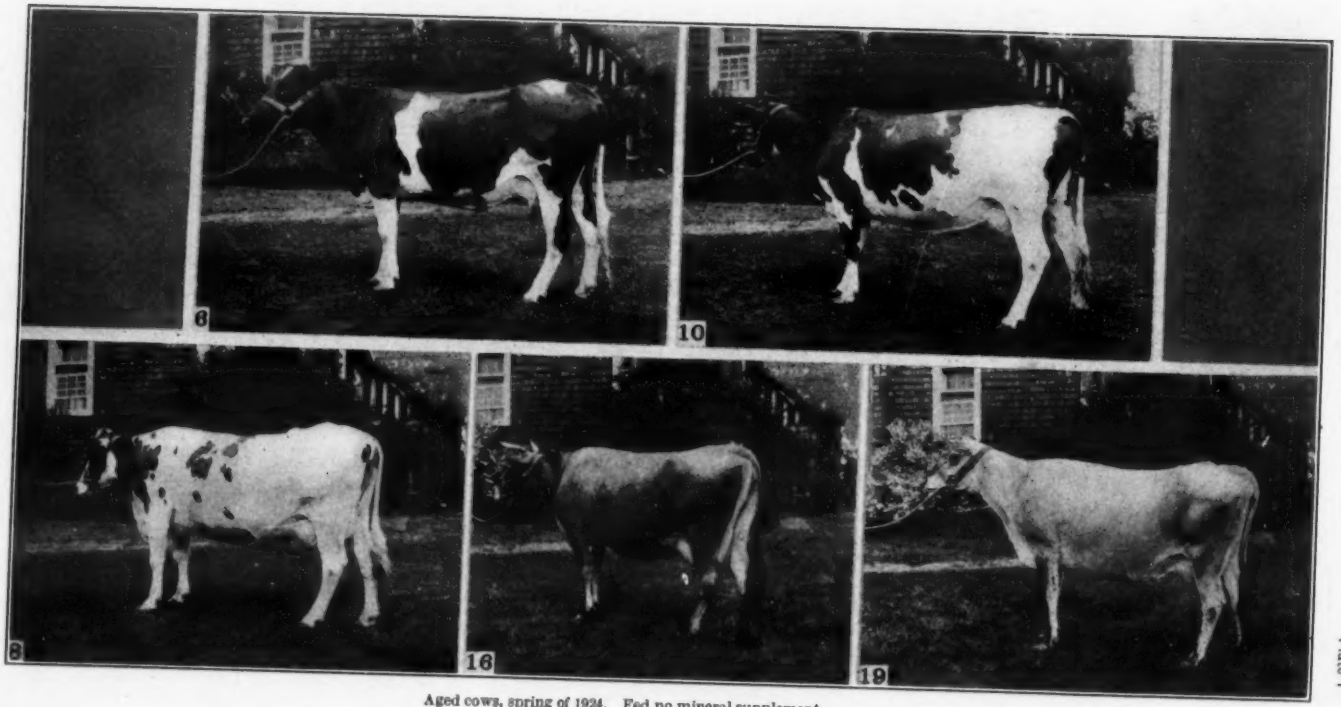
Another group of 6 individuals consists of 6 young cows which were either heifers or calves when the experiment commenced. (See Table II.)

TABLE II.—*History and present condition of the young cows*

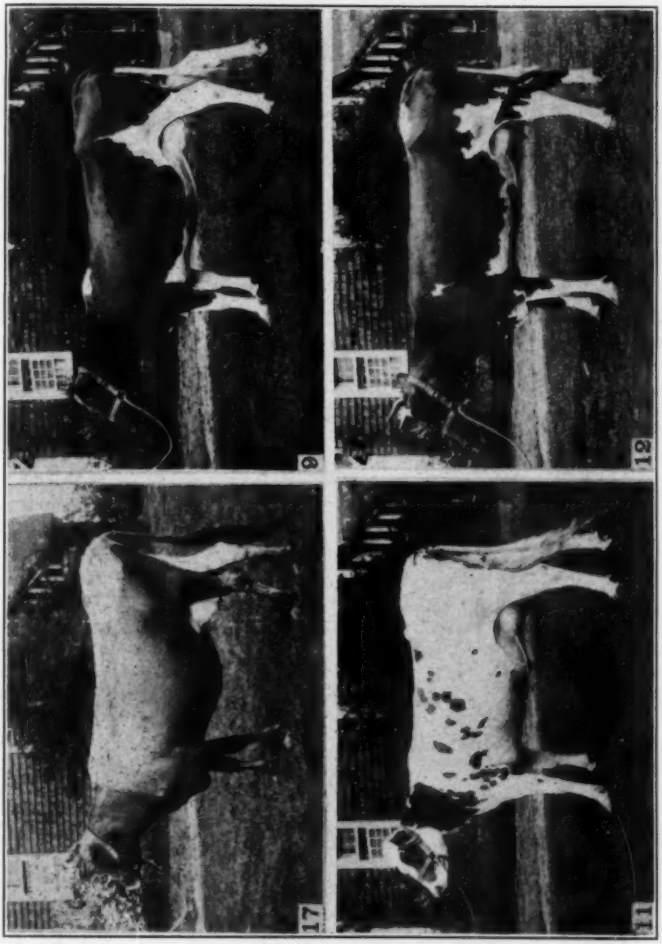
Cow No.	Age in years	Breed	Group	Number of calves		Present condition (May, 1924)
				Previous to commencement of experiment	Since commencement of experiment	
3	3	Grade Holstein	Mineral	None.	1	Fair.
5	3	do	Nonmineral	None.	1	Good.
15	5	do	do	[a] None.	2	Do.
22	4	Grade Jersey	Mineral	None.	1	Poor.
23	4	do	Nonmineral	None.	2	Very poor
24	3	do	Mineral	None.	2	Poor.

[a] All except No. 15 too young to breed; No. 15 had been bred but had not calved.

Here the situation is somewhat different. The last three cows in the list (pl. 3) are without a doubt suffering from lack of something in their ration, and that something is presumably calcium. It is perhaps



Aged cows, spring of 1924. Fed no mineral supplement



Aged cows, spring of 1924. Fed mineral supplement

significant that all three are Jerseys and that two of them have received the mineral supplement. The three Holsteins, on the contrary, have maintained themselves in a good state of nutrition, and it is rather paradoxical that the two that have not received any mineral supplement are in excellent condition, as good as any of the cows in the herd (pl. 3).

A third group consists of five heifers born since the commencement of the experiment. (See Table III.)

TABLE III.—*History and present condition of the heifers*

Heifer No.	Age in months	Breed	Group	Present condition (May, 1924)
40	21	Grade Holstein	Mineral	Good.[a]
42	19	do	Nonmineral	Do.
43	19	Purebred Jersey	do	Excellent.[b]
57	9	Grade Holstein	do	Fair.[b]
59	8	Jersey-Ayrshire cross	do	Poor.[b]

[a] Recently bred. [b] Unbred.

The last two of these are too young to pass judgment on, at least from this standpoint. As the progeny of cows in the experiment they will be discussed later on. The first three apparently have not as yet felt any bad effects of the low ash rations, nor has the mineral supplement added to No. 40's ration had any perceptible effect. She and her mate (No. 42) are, to all outward appearances, in identical condition (pl. 4).

The question will probably be raised as to why there are four heifers in the "nonmineral" group and only one in the "mineral" group. The procedure which, with a few exceptions, has been followed, has been to place the heifer calves in the same group as their dams, to see if any effects of the experiment might be cumulative. This past year, due to unfortunate circumstances in no way connected with the experiment, three heifer calves from cows in the "mineral" group have died, thus making an uneven division of the young animals. This fault, if not corrected during the present season by natural increase, will be adjusted by placing calves from "nonmineral" cows in the "mineral" group.

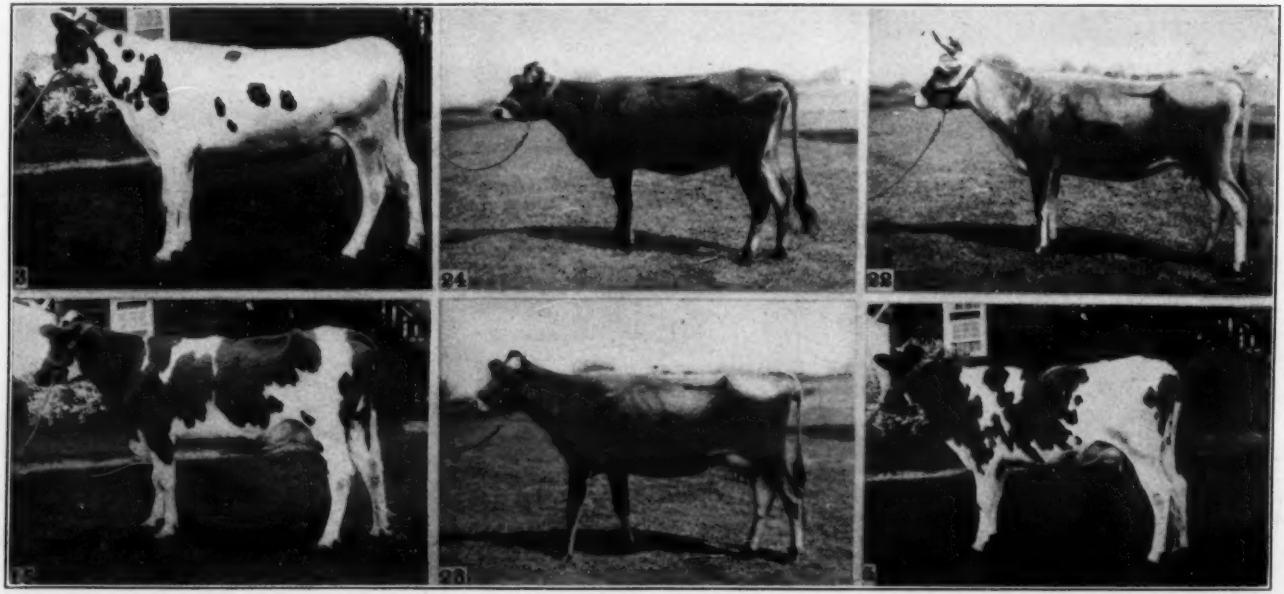
Summing up the evidence on all the individuals it is found, as far as general appearance and handling are concerned, that—

(1) The experimental treatment has had no prolonged ill effects on the aged cows, irrespective of breed.

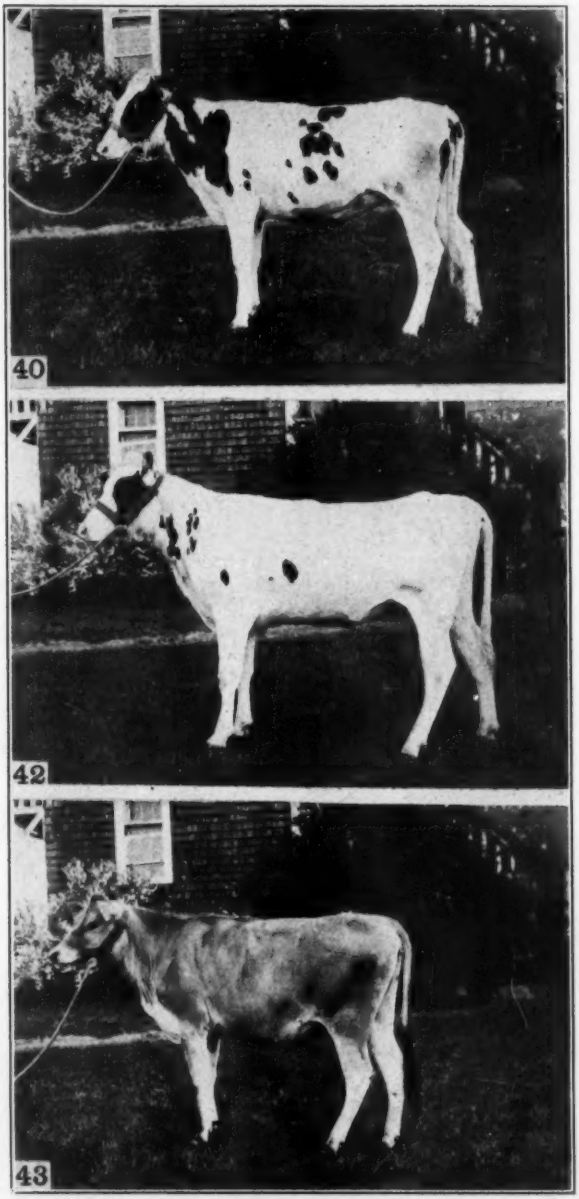
(2) The same is true so far of the heifers and of the young Holstein cows.

(3) The young Jersey cows, irrespective of the group they belong to, show conclusive evidence of impaired metabolism, in their general unthrifty appearance, thin flesh, and rough, harsh coats.

Not satisfied with their own judgment in the matter, the writers had the opinion of 10 impartial outsiders, all more or less experienced in the judging of cattle. A careful record of their decisions has been kept, and an analysis of this record shows that their decisions averaged 58 per cent correct, i. e., they had almost as many cows in the wrong group as in the right one. The mistakes were almost exactly divided between the two groups, 57.2 per cent of the mineral group



Young cows, spring of 1924. Nos. 3, 24, and 22 were fed mineral supplement. Nos. 15, 23, and 5 were not fed mineral supplement



Heifers, spring of 1924. No. 40 was fed mineral supplement. Nos. 42 and 43 did not receive mineral supplement

being rightly placed and 58.6 per cent of the nonmineral group. All agreed that, with two or three exceptions, there was no significant difference in the general appearance and handling of the cows.

Body Weight

For obvious reasons, only those cows which were mature at the beginning of the experiment can be considered under this heading. There are seven such individuals in the herd, and an endeavor has been made to have the weight for each of these at the beginning of the experiment and during the present season as comparable as possible. This has been done by noting the stage of lactation and gestation of each cow when the experiment started and by fixing a date during this present year at which time she was in approximately the same stage of gestation and lactation. An average of four separate weighings, one each on the first and second days of two consecutive months, has been taken in each instance. (See Table IV.)

Table IV.—*Net gain or loss in weight of the aged cows*

Cow No.	At beginning of experiment				Date this season when cow was at approximately the same stage of lactation and gestation	Average weight at that time	Gain or loss
	Age in years	Average weight in pounds	Stage in lactation in months	Stage in gestation in months			
Nonmineral group:							
8	9	1,215	4	(a)	June, 1924	1,235	+20
10	7	1,100	3	1	December, 1923	1,085	−15
16	8	910	11	6	February, 1923 [b]	920	+10
19	5	890	2	(a)	December, 1923	860	−30
Net gain or loss for the group							−15
Mineral group:							
9	7	1,230	3	1	October, 1923	1,205	−25
11	7	1,210	4	1	March, 1924	1,285	+75
17	9	870	2	(a)	December, 1923	850	−20
Net gain or loss for the group							+30

[a] Not bred.
[b] Can not get this cow with calf this year, so her weight a year ago is taken.

Because of the considerable variation in weights of cows, even from day to day, such small differences as noted above can not be considered of any significance.

Milk Production

As noted elsewhere in this paper, Meigs (*22*) is of the opinion that cows fed rations deficient in mineral matter react to the deficiency by a marked decrease in milk flow from year to year.

The effect of the experiment in this respect has been noted by a careful study of the production records obtained by the writers. The results are presented in the accompanying charts, which show the average daily milk yield per cow by months, for the whole herd since January, 1920, and for the two groups since the commencement of the experiment in December, 1921.

It is quite evident (Chart I) that thus far the low-ash rations have had no appreciable effect on the milk yield of the herd as a whole. Also, it is equally evident (Charts II and III) that the mineral supplement has had no effect. The average daily milk yield of the cows in the mineral group has been slightly lower all through the

experiment than that of the cows in the nonmineral group. This can be ascribed in part to the slightly lower ratio of Holsteins to Jerseys in the mineral group, but computation shows that this is only a minor factor, and the lower yield must be considered as due chiefly to differences in the inherent ability of the individual cows to produce milk.

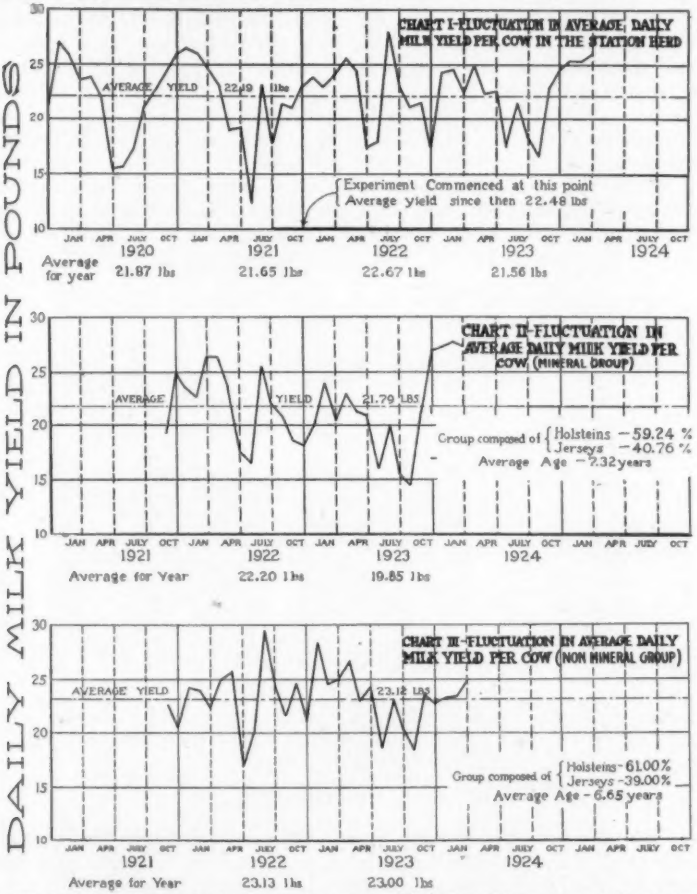

DAILY MILK YIELD IN POUNDS
CHART I-FLUCTUATION IN AVERAGE DAILY MILK YIELD PER COW IN THE STATION HERD
AVERAGE YIELD 22.49 lbs
Experiment Commenced at this point
Average yield since then 22.48 lbs
1920 1921 1922 1923 1924
Average for year 21.87 lbs 21.65 lbs 22.67 lbs 21.56 lbs
CHART II-FLUCTUATION IN AVERAGE DAILY MILK YIELD PER COW (MINERAL GROUP)
AVERAGE YIELD 21.79 LBS
Group composed of Holsteins - 59.24 %
Jerseys - 40.76 %
Average Age - 7.32 years
1921 1922 1923 1924
Average for Year 22.20 lbs 19.85 lbs
CHART II-FLUCTUATION IN AVERAGE DAILY MILK YIELD PER COW (NON MINERAL GROUP)
AVERAGE YIELD 23.12 LBS
Group composed of Holsteins - 61.00%
Jerseys - 39.00%
Average Age - 6.65 years
1921 1922 1923 1924
Average for Year 23.13 lbs 23.00 lbs

FIG. 1.—Charts showing fluctuation in the average daily milk yield per cow—for the station herd as a whole, for the group which was fed mineral supplement, and for the group which did not receive mineral supplement

COMPOSITION OF THE MILK

As already noted, five-day composite samples of each cow's milk have been taken during each month she has been in milk. Total solids and fat have been determined regularly on these samples. The total ash of the samples has been determined in 12 different months extending over a period from June, 1922, to March, 1924. The calcium and phosphorus of the ash have been determined in nine different months extending over a period from October, 1922, to March, 1924. The results are presented in Table V.

TABLE V.—*Composition of milk*

Period	Total solids	Fat	Total ash	Amount of ash in total solids	Calcium	Phosphorus
Preexperimental period, Jan., 1920, to Nov., 1921, whole herd	*Per cent* 13.44	*Per cent* 4.83	*Per cent* (a)	*Per cent* (a)	*Per cent* (a)	*Per cent* (a)
Experimental period, Dec., 1921, to Apr., 1924, whole herd	13.23	4.52	0.722	5.38	0.119	0.099
Mineral group	13.40	4.62	.721	5.28	.122	.099
Nonmineral group	13.06	4.42	.723	5.48	.117	.099

a Not determined.

The average content of total solids and fat has been slightly lower since the commencement of the experiment than it was during the two years previous, but this can be accounted for by the fact that since the experiment commenced the number of analyses of Jersey milk has been 6.94 per cent less than in the preexperimental period, while the analyses of Holstein milk have been 4.80 per cent greater. Due allowance having been made for this difference, it is seen that, taking the herd as a whole, the percentages of total solids and fat in the milk have not been materially affected by the conditions of the experiment.

The average for the mineral group is slightly higher than for the nonmineral group, but a similar reason to that given above accounts for the difference. While the number of Jersey milks tested was identical for each group, there were 14.6 per cent more Holstein milks from the nonmineral group than from the mineral group, which explains the somewhat lower fat and solid test of the former.

Respecting total ash and calcium and phosphorus, it is seen that with possibly one exception variations between the two groups are so slight as to be of no significance. The exception is calcium, which is slightly higher in the milks from the mineral group. It is possible that this may be due to the larger amount of calcium which the mineral group has received, but it is also possible that it may be due to inherited tendencies of the individual animals. As the figures are averages of 106 calcium determinations on milks from 18 different cows, it is unlikely that the difference is due to experimental or analytical error.

EFFECT ON REPRODUCTION

Under this heading have been observed:

(1) Recurrence of oestrum with respect to regularity and to the interval elapsing after calving before reappearance of oestrum. Regarding regularity, there are 19 individuals for which data are available. Scrutiny of their records reveals the following:

Regularity	Total	Mineral group	Nonmineral group
Good	13	6	7
Fair	5	3	2
Poor	1	1	0

In respect to the interval of time elapsing after calving before reappearance of oestrum, the average number of days throughout the course of the experiment has been:

	Days
Mineral group	39. 5
Nonmineral group	38. 6

These data show that both groups were quite similar in their behavior in these respects; in fairness, however, it must be stated that as the experiment has progressed the mineral group of cows has tended to come in heat sooner after calving, while the reverse has been true of the nonmineral group.

(2) Difficulties in getting cows bred. Considerable trouble has been experienced in getting the cows with calf, some of them having to be served four or five times before they would hold. The trouble has become somewhat more prevalent as the experiment has progressed, but has been quite evenly divided between the two groups, as shown by the accompany figures:

Group	Average number of times bred		
	1922	1923	1924
Mineral	1. 60	2. 25	2. 50
Nonmineral	1. 67	2. 67	2. 25

(3) Other abnormalities. There have been three cases of abortion and one premature birth in the herd since the commencement of the experiment. Two of the abortions were in the mineral group; the other one and the premature birth were in the nonmineral group. Two of the abortions are known to have been traumatic, and the others probably can not be attributed to the effect of the experiment.

There have been two cases of retained placenta, both in the nonmineral group; but one of them was in the case of a cow which had had the same trouble previous to commencement of the experiment.

(4) Condition of the calves at birth and subsequently. All calves dropped during the course of the experiment have either been raised to maturity or kept a sufficiently long time to enable an accurate opinion to be formed as to their constitutional vigor. Table VI summarizes the records.

TABLE VI.—*Condition of calves*

Group	Vigorous	Good	Fair	Delicate	Died at birth	Abortions
Mineral:						
1922	1	1		1		1
1923	2		2	1	1	1
1924		1	2			
Total	3	2	4	2	1	2
Nonmineral:						
1922	1	1	2	2		
1923	2	2	2	1	1	1
1924	1			2		
Total	4	3	4	5	1	1

Relatively, each group has produced about the same proportion of vigorous and good calves. The mineral group has had a somewhat higher proportion of fair calves, while the nonmineral group has had relatively almost twice as many delicate ones. The deaths at birth and the abortions probably have no bearing on the experiment, but are presented in order that the statistics may be complete. It is perhaps significant that there have been a relatively large number of delicate calves from the nonmineral group. The weakness of constitution has manifested itself for the most part in a very delicate and sensitive digestive system. All of these delicate calves had to be pampered along, not even making satisfactory progress on a whole milk–skim milk diet, and in the end every one of them died or had to be killed. Post-mortem examination revealed in every instance more or less inflammation of the digestive tract, amounting in at least one case to a chronic gastrointestinal catarrh.

It would be unwise, however, to attach too much weight to this evidence, for other factors, such as the individuality and breed of the dams, has no doubt had considerable to do with the condition of the calves. All the vigorous and good calves have been from Holstein cows. All the fair and delicate ones, with four exceptions, have been from Jersey cows. Three of the four exceptions were heifer's calves, and were not sired by a Holstein bull.

SUMMARY

The object of this experiment has been to test the efficacy of adding supplemental inorganic salts of calcium to the rations of dairy cows.

The whole station herd has been fed for two and a half years on a ration decidedly low in calcium, too low, from the standpoint of our present knowledge, for the needs of the animals. One-half of the herd has had the deficiency made good theoretically by supplementing the ration with calcium phosphate in the form of special steamed bone meal.

The following are the outstanding facts revealed by the investigation thus far:

(1) Irrespective of the group to which they belong, the experimental procedure has had no prolonged ill effects on the general condition of the aged cows, of the young Holstein cows, or of the heifers, but has apparently seriously disturbed the metabolism of the young Jersey cows.

(2) Changes in body weight of the aged cows have been insignificant. Obviously, the young cows and heifers can not be considered under this heading.

(3) Milk production has not been significantly affected either by the low-calcium ration or by the mineral supplement, except in the case of the young Jersey cows. They have not milked as freely or as persistently as it was expected they would. Too much stress, however, should not be placed upon this latter statement, because of the small number of cows studied.

(4) With the possible exception of calcium, the composition of the milk has not been appreciably effected.

(5) The reproductive function has been more seriously disturbed than any other, considerable difficulty being experienced in getting

the cows with calf. This difficulty has increased as the experiment has progressed, but has been about equally divided between the two groups.

(6) Each group has produced about the same proportion of strong, healthy calves. The nonmineral group has had much the higher proportion of delicate calves, but this is due in part to breed characteristics and should not be taken too seriously.

(7) It should be borne in mind that this summary is a report of progress and should not be considered as final.

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THE WATER-SOLUBLE VITAMIN CONTENT OF THE VELVET BEAN [1]

By W. D. SALMON, *Assistant in Animal Industry Research*, and EMERSON R. MILLER, *Research Chemist, Agricultural Experiment Station of the Alabama Polytechnic Institute*

INTRODUCTION

Brief studies of the water-soluble vitamin content of the velvet bean have been reported. Data collected at the Agricultural Experiment Station of the Alabama Polytechnic Institute in 1920–21 by Miller,[2] Burleson, and Templeton show that velvet beans or alcoholic extracts of velvet beans improved the condition of polyneuritic pigeons.

Salmon [3] observed that polyneuritic pigeons were improved by the administration of ground velvet beans, and also that the onset of polyneuritis was delayed by furnishing an aqueous extract of velvet beans as the sole source of water to pigeons receiving a diet of polished rice.

Sure and Read [4] report that when rats received 60 to 80 per cent of autoclaved velvet beans, or 40 per cent of the raw beans, as the source of vitamin B, little growth resulted. These investigators concluded that the velvet bean is very low in vitamin B.

These results led the writers to undertake a more complete investigation in which velvet beans from the same source would be tested on both pigeons and rats.

CURATIVE EXPERIMENTS WITH PIGEONS

A number of pigeons that had developed pronounced symptoms of polyneuritis on a diet of polished rice were restored to an apparently normal condition by 1 to 2 grams of finely ground velvet beans.[5] One pigeon (No. 19) that was having spasms was given 1 gram of velvet beans. The bird appeared to be normal the next day, and no further symptoms of polyneuritis appeared for five days after the 1 gram of beans was administered.

Other pigeons were cured by alcoholic and acetic-acid extracts of velvet beans. Several of the polyneuritic pigeons that received such extracts were not cured, however. In most cases considerable improvement in the condition of the bird was noted, and in a few cases birds that had apparently been cured died a few days later.

PREVENTIVE EXPERIMENTS WITH PIGEONS

The preventive tests were begun in July, 1922, with velvet beans from the 1921 crop. The beans or extracts of the beans were fed by hand. Polished rice was kept before the birds at all times, except in the cases where it was fed by hand (noted in Table I).

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[1] Received for publication Jan. 26,1925; issued December, 1925. Published with the permission of the director of the Alabama Experiment Station.

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[5] Shelled velvet beans, Early Speckled variety, were used in all tests reported in this paper.

TABLE I.—*Record of pigeons by periods*

	Length of period	Initial weight	Gain (+) or loss (−)	Average daily feed		
				Rice	Beans	Extract
Pigeon No. 8:	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Period A	41	299	−86	[a] 11.4	----------	----------
Period B	49	213	+28	17.3	----------	[b] 0.50
Period C	21	241	−18	10.0	----------	[c] 0.25
Period D	35	223	+9	[a] 12.1	5.0	----------
Period E	42	232	+38	[a] 16.3	2.0	----------
Pigeon No. 1:						
Period A	7	320	−30	(?)	----------	----------
Period B	63	290	−17	16.3	----------	[d] 0.38
Period C	70	273	+7	13.5	2.0	----------
Period D	14	280	−43	6.0	[e] 2.0	----------
Period E	77	287	+5	12.0	1.0	----------
Pigeon No. 14:						
Period A	70	278	−50	16.5	----------	[f] 0.67
Period B	70	228	+22	19.0	2.0	----------
Period C	21	250	−80	15.7	[e] 2.0	----------
Pigeon No. 13:						
Period A	77	268	−13	[a] 12.3	4.67	----------
Period B	28	255	+8	[a] 16.5	1.80	----------
Pigeon No. 17	17	323	−130	[a] 11.4	[e] 4.0	----------
Pigeon No. 3:						
Period A	19	304	−74	19.4	----------	----------
Period B	7	230	−23	[a] 17.0	----------	----------
Pigeon No. 4:						
Period A	19	301	−73	21.0	----------	----------
Period B	17	228	−9	[a] 15.5	----------	----------
Pigeon No. 7	5	266	−87	----------	9.0	----------
Pigeon No. 11	9	264	−53	----------	11.1	----------

[a] Polished rice hand fed.
[b] First percolate from 8 grams beans.
[c] Flask extraction from 2.77 grams beans.
[d] Second percolate from 4 grams beans.
[e] Velvet beans extracted by alcohol.
[f] Second percolate from 3.2 grams beans.

Table I and Figure 1 show a summary of the results. When alcoholic extracts of velvet beans were given to pigeons that were declining in weight on a diet of polished rice, the decline was checked and in some cases gains in weight occurred (periods B and C of No. 8, A of No. 14, and B of No. 1). Pigeon No. 8 developed polyneuritis on a diet of polished rice in period A. A daily dose of alcoholic extract [6] representing 8 grams of velvet beans was then given throughout period B. The bird was restored to an apparently normal condition and made decided gains in this period. The bird was then changed to a different alcoholic extract [7] in daily doses representing 2.77 grams of beans. The bird received this extract for only 21 days (period C). There was considerable loss of weight, but the pigeon was apparently in good condition at the close of the period.

That the extract given No. 8 in period B did not carry all the protective substance contained in the beans is shown by period A of No. 14 and period B of No. 1.

After the percolate had been removed for the preparation of the extract given No. 8 in period B, the percolation was continued and an extract prepared from the second fraction. Pigeons Nos. 1 and 14 received this second fraction extract representing 4 grams and 3.2 grams of velvet beans per day, respectively, for 70 days. Both birds declined in weight considerably, but did not show any symptoms of polyneuritis. The longest-surviving check birds (Nos. 3 and 4) lived only 26 and 36 days, respectively, after they were started on polished rice.

[6] This extract was prepared by percolating ground velvet beans with cold 90 to 95 per cent alcohol, and evaporating the percolate on dextrin.
[7] This extract was prepared by extracting velvet beans on boiling water bath with four fractions of 90 to 95 per cent alcohol, each fraction being heated two hours.

In these tests raw velvet beans were more efficient than the extracts. Daily doses of 2 grams of the raw beans gave complete protection and enabled birds weighing 220 to 270 grams to maintain their weight or make slight gains through periods of 40 to 70 days (periods D and E of No. 8, A and B of No. 13, C of No. 1, and B of No. 14).

Velvet beans that had been extracted with alcohol in Soxhlet for 48 hours did not have any protective action (pigeon No. 17, and periods C of No. 14 and D of No. 1). Pigeon No. 17 received 4 grams of the extracted material per day, and pigeons Nos. 1 and 14

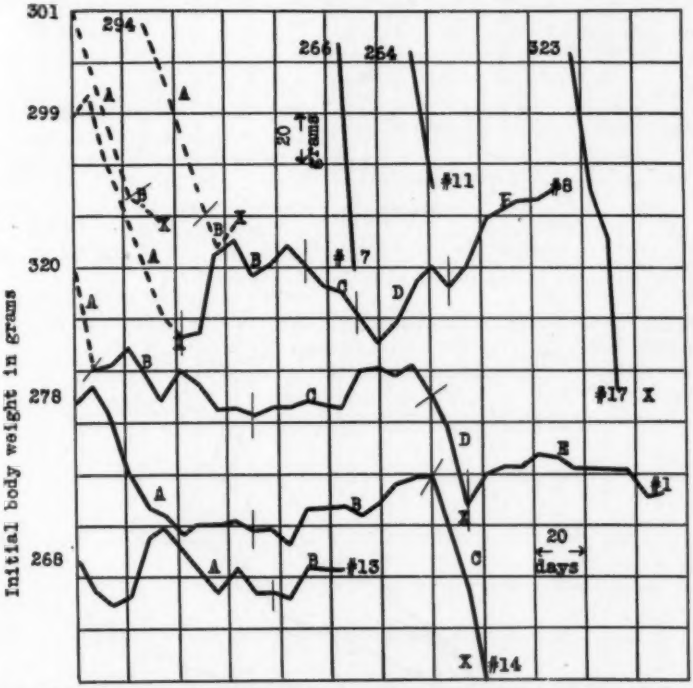

FIG. 1.—Weight curves of pigeons: Effect of feeding velvet beans. Letters refer to the periods in Table I Broken lines indicate periods in which polished rice alone was fed. X indicates onset of polyneuritis

received 2 grams each per day. All three birds lost weight rapidly and came down with polyneuritis in 14 to 21 days.

After pigeon No. 1 was unable to stand, it was given 2 grams of raw velvet beans, and it appeared to be almost normal the next day. This bird was again given 2 grams of raw beans on the second day, and then 1 gram per day for 77 days (period E). No symptoms of polyneuritis appeared during this period.

Pigeons Nos. 7 and 11 received raw velvet beans alone, hand fed (no rice). They lost weight rapidly and died on the fifth and ninth days, respectively. The harmful effect of a diet of velvet beans alone has previously been noted.[8]

[8] SALMON, W. D. THE EFFECT OF FEEDING VELVET BEANS TO PIGEONS. Science 56: 368. 1922.

GROWTH EXPERIMENTS ON RATS

The usual laboratory routine for vitamin B tests was followed in these experiments. Young white rats were caged individually on screens which prevented access to excreta. The rats were fed once each day and weighed once a week. A record of feed consumption was kept.

The following basal diet was fed until the rats were stationary or declining in weight:

	Per cent
Casein [9]	18. 0
Salts (McCollum's No. 185)	3. 7
Agar	2. 0
Rice powder [10]	71. 3
Butter fat [11]	5. 0

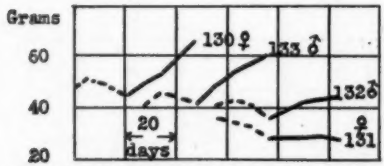

FIG. 2.—Weight curves of rats. Basal diet, plus 10 per cent raw velvet beans. Broken lines in charts 2 to 10 represent periods in which the basal vitamin B free diet alone was used

The velvet beans or extracts were then added to the basal diet, replacing an equal amount of rice powder. When yeast was added, an amount of dried yeast [12] equal to 5 per cent of the daily allowance of feed was placed on top of the other feed in the feeder.

The addition of 10 per cent of raw velvet beans to the basal diet checked the decline of rats and enabled them to make very slight gains (fig. 2).

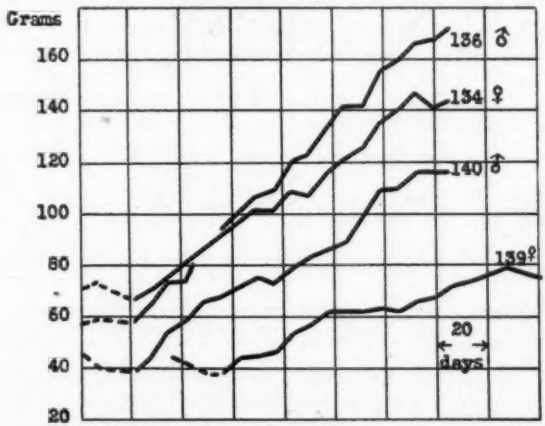

FIG. 3.—Weight curves of rats. Basal diet, plus 20 per cent raw velvet beans

When 20 per cent of raw beans was added to the basal diet the rats grew at approximately one-third of the normal rate (fig. 3).

When 5 per cent of dried yeast was added to the above diet normal growth occurred (fig. 4). The females were bred, and then littered

[9] Washed one week in 0.2 per cent acetic acid.
[10] Heated in electric oven five hours at 120° C.
[11] Thoroughly washed and centrifuged.
[12] Dried yeast (Harris).

young that were nearly normal in weight but raised none of them to weaning. After the addition of 2.5 c. c. of orange juice per day to the diet, female No. 71 raised part of the next litter, but the young did

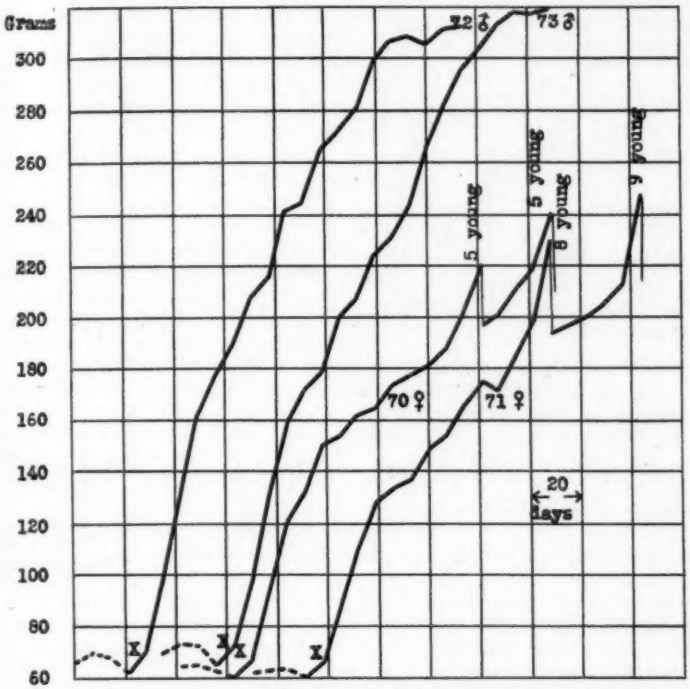

FIG. 4.—Weight curves of rats. Basal diet, plus 20 per cent raw velvet beans. At point X, 5 per cent of dried yeast was added

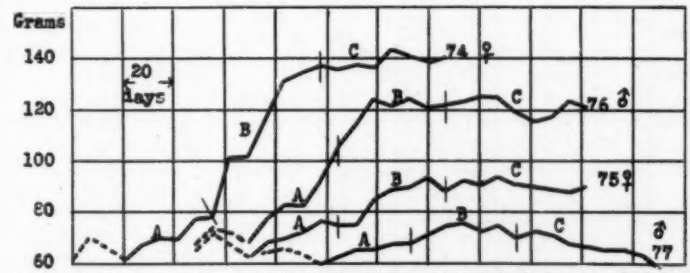

FIG. 5.—Weight curves of rats. Basal diet, plus 20 per cent cooked velvet beans. Periods A and C; 20 per cent raw velvet beans Period B

not make normal growth. After the addition of 10 c. c. of whole milk per day, female No. 70 raised the second litter successfully, and the young grew to maturity at the normal rate on the diet of the mother.

Velvet beans cooked on boiling-water bath for one and one-half hours were no more efficient as a source of vitamin B than raw velvet beans, when both were fed at a concentration of 20 per cent (fig. 5).

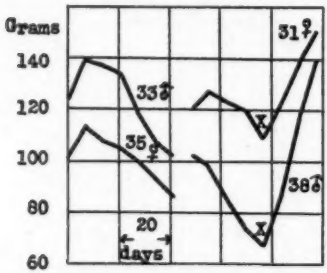

FIG. 6.—Weight curves of rats. Basal diet, plus 20 per cent velvet beans previously extracted with alcohol. At point X, 5 per cent dried yeast was added

Rats lost weight rapidly on the basal diet plus 20 per cent of velvet beans that had been extracted with alcohol in Soxhlet for 24 hours. The addition of 5 per cent of yeast to this diet enabled the rats to resume growth (fig. 6).

When 50 per cent of raw velvet beans was added to the basal diet there was no appreciable growth (fig. 7). Growth was even less satisfactory on this diet than on the one that contained 10 per cent of raw velvet beans. Apparently the benefit of the added supply of vitamin was overcome by the injurious effect of the larger percentage of beans. The addition of 5 per cent of yeast to the diet that contained 50 per cent of raw beans resulted in slight improvement in the growth rate (fig. 8), but did not produce as satisfactory growth as the addition of a like amount of yeast to the diet that contained only 20 per cent of raw beans. There was considerable diarrhea among the rats that received the diet containing 50 per cent of raw beans.

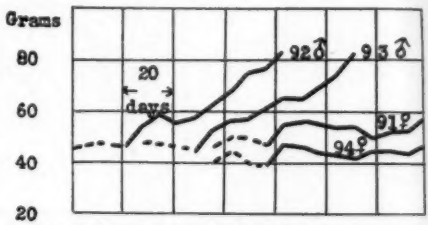

FIG. 7.—Weight curves of rats. Basal diet, plus 50 per cent raw velvet beans

The results indicate that the harmful effect of the beans was the most important limiting factor in this diet, although the 50 per cent of raw beans apparently did not furnish the optimum supply of vitamin B.

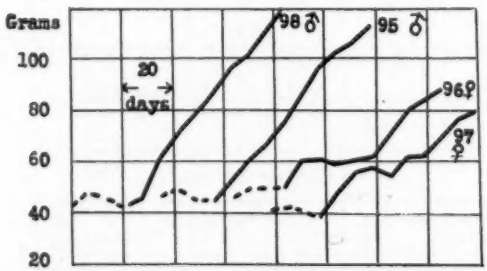

FIG. 8.—Weight curves of rats. Basal diet, plus 50 per cent raw velvet beans, plus 5 per cent yeast

A comparison of Figures 8 and 9 shows that cooking the beans on boiling-water bath for one and one-half hours lessened the harmful effect. The feces of the group receiving 50 per cent of cooked beans (with yeast) were normal, and the rats remained clean and plump, in marked contrast to the soiled, unthrifty appearance of those that received 50 per cent of raw beans. Growth was not as satisfactory, however, on 50 per cent of cooked beans (yeast added) as on 20 per cent of raw beans (yeast added).

Autoclaving the beans for two hours at 15 pounds pressure lessened their harmful effect but did not give better results than cooking on the water bath.

Various alcoholic and acetic-acid extracts were made in an effort to obtain the growth factor free from the harmful substance. These extracts were added to the basal diet to represent varying percentages of beans, but in all cases the extracts were less potent than the same percentage of raw beans.

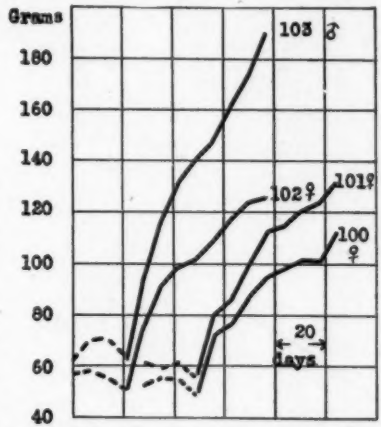

FIG. 9.—Weight curves of rats. Basal diet, plus 50 per cent cooked velvet beans, plus 5 per cent yeast

SUMMARY

Polyneuritic pigeons were restored to apparently normal health by raw velvet beans and by alcoholic and acetic-acid extracts of velvet beans. Pigeons weighing 220 to 270 grams and receiving a diet of polished rice were protected against the onset of polyneuritis by 2 grams of raw velvet beans per day. This amount represented 9.5 to 13 per cent of the total daily feed. Velvet beans that had been thoroughly extracted with alcohol had no protective action when fed in doses of 2 to 4 grams per day.

In one case, 1 gram of raw velvet beans per day prevented the onset of polyneuritis during a period of 77 days' feeding on polished rice.

Although small amounts of velvet beans had a protective action, large amounts were harmful. This was shown by the death of two pigeons in five and nine days, respectively, when they were fed beans alone.

When raw velvet beans were used as the sole source of vitamin B, rats grew more rapidly on diets that contained 20 per cent of beans than on diets that contained either 10 per cent or 50 per cent of beans.

The addition of 5 per cent of yeast to diets carrying 20 per cent or 50 per cent of raw beans caused a marked increase in the rate of growth. The effect was more pronounced, however, in the case of diets carrying the 20 per cent concentration of beans.

Cooking velvet beans one and one-half hours on boiling water bath lessened but did not entirely overcome their harmful effect on rats.

Autoclaving the beans for two hours at 15 pounds pressure was no more beneficial than cooking them as described above.

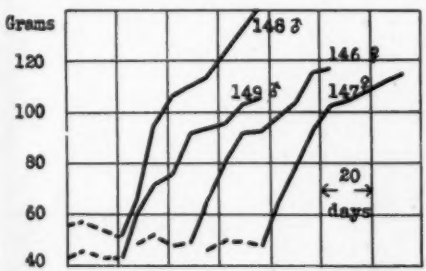

FIG. 10.—Weight curves of rats. Basal diet, plus 50 per cent autoclaved velvet beans, plus 5 per cent yeast

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